Ginsenoside Rg1 alleviates A β deposition and neuronal damage by inhibiting NLRP1 inflammasome activation in APP/PS1 mice

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Research

Keywords: Ginsenoside Rg1, APP/PS1 mice, NLRP1 inflammasome, NADPH oxidase 2, Alzheimer's disease

Posted Date: August 2nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-49467/v1>

Abstract

Background: Oxidative stress and neuroinflammation play important roles in the whole pathogenesis of Alzheimer's disease (AD). NADPH oxidase 2 (NOX2) is an important enzyme that is responsible for ROS generation in many neurodegenerative diseases. The nucleotide-binding oligomerization domain (NOD)-like receptor protein 1 (NLRP1) inflammasome is responsible for the formation of pro-inflammatory molecules in neurons. However, it is still unclear whether inhibition of NOX2 and NLRP1 inflammasome decreases amyloid-beta (A β) generation and deposition in APP/PS1 mice. Ginsenoside Rg1 (Rg1) is an active component in ginseng, and maybe a potential agent for neurodegenerative diseases. In this study, we investigated the effects and mechanisms of Rg1 treatment on cognitive performance, neuronal damage, A β deposition and NOX2-NLRP1 inflammasome activation in APP/PS1 mice.

Methods: WT and APP/PS1 mice were used in the experiment from 6 months (M) old to 9M old, and 6M APP/PS1 mice were used as pre-treatment controls. The open field test (OFT) and Morris water maze (MWM) were used to detect behavioral change and cognitive function. The H&E and Nissl staining were used to assess neuronal damage. We further examined PSD95 expression, A β generation and deposition, Tau and p-Tau expression, NOX2-mediated ROS generation and NLRP1 inflammasome activation by using immunofluorescence, western blot analysis, and real time q-PCR.

Results: Rg1 treatment for 12 weeks alleviated learning and memory impairments and neuronal damage, decreased p-Tau level, APP expression and A β deposition in APP/PS1 mice. Meanwhile, Rg1 treatment significantly decreased the levels of ROS and IL-1 β , and reduced the expressions of NOX2 and NLRP1 inflammasome in the brain cortex and hippocampus in APP/PS1 mice. Furthermore, apocynin (a NOX inhibitor) and NLRP1-siRNA treatment also alleviated cognitive impairments, neuronal damage and A β deposition, and reduced the expression of NLRP1 inflammasome in brain cortex and hippocampus in APP/PS1 mice.

Conclusions: Rg1 treatment could alleviate learning and memory impairment, neuronal damage, and reduce A β generation and deposition by inhibiting NLRP1 inflammasome activation in APP/PS1 mice.

Highlights

- The expressions of NOX2 and NLRP1 inflammasome were significantly increased in APP/PS1 mice.
- Rg1 treatment significantly alleviated cognitive dysfunction, A β deposition and neuronal damage in APP/PS1 mice.
- Rg1 treatment significantly decreased NOX2 expression and ROS accumulation in APP/PS1
- Rg1 treatment significantly reduced NLRP1 inflammasome expression and IL-1 β level in APP/PS1

Background

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss and cognitive decline. Clinically, the main pathological features of AD are the extracellular accumulation of senile plaques comprised of amyloid-beta (A β) peptides, intracellular neurofibrillary tangle composed of highly phosphorylated Tau (p-Tau) and neurodegenerative changes accompanied by synaptic dysfunction and neuronal damage [1]. Currently, the pathogenesis of AD is still not completely understood. Increasing evidence suggests that neuroinflammation plays important role in the pathogenesis and the behavioral disturbances associated with AD [2-4]. Although a large number of epidemiological researches have proven that the traditional non-steroidal anti-inflammatory drugs (NSAIDs) could inhibit neuroinflammation and alleviate the severity of cognitive impairment, gastrointestinal complications limited the long-term use of these drugs [5]. At present, there are still no effective drugs for preventing the generation and progression of AD. Thus, the development of effective drugs with limited or no side effects is necessary for AD treatment.

In recent years the natural medicines with fewer side effects and safer performance have been widely used to treat many diseases including AD [6, 7]. Ginseng has been safely used in China for more than 2000 years as it improves health conditions and delays senescence [8]. Ginsenoside Rg1 (Rg1), one of the main active components of ginseng, is a tetracyclic triterpenoid saponin [9]. It has been reported to be a potential neuroprotective agent in several animal models of neurological diseases and cognitive impairments [10, 11]. Our previous study demonstrated that Rg1 was able to alleviate H₂O₂-induced neuronal damage by inhibiting NADPH oxidase 2 (NOX2) and nucleotide-binding oligomerization domain (NOD)-like receptor protein 1 (NLRP1) inflammasome activation in hippocampal neurons in vitro [12]. These data suggested that Rg1 treatment might delay the occurrence and development of AD through its antioxidant and anti-inflammatory effects. Therefore, it is important to study the protective effects and precise mechanisms of Rg1 on APP/PS1 AD mice.

It has been reported that oxidative stress plays an important role in the pathogenesis of many neurodegenerative diseases. Excessive reactive oxygen species (ROS) generation can not only induce oxidative stress damage but also activate an inflammatory response, which has been considered to contribute to the pathogenesis and progression of AD [13]. NADPH oxidases (NOXs) are the main enzymes that contribute to excessive ROS generation in many tissues and also involved in the development of the AD pathological process [14]. NOX2 is found to be constitutively expressed in the brain, especially in neurons [15]. A recent study suggested that NOX2 contributed to aging-related neuronal oxidative stress damage and loss of brain function [16]. Our previous research indicated that the expressions of NOX2 and its subunits of Rac1, p47phox and p22phox were significantly increased in long-term cultured hippocampal neurons [17]. Meanwhile, treatment with Rg1 significantly decreased the expressions of NOX2 and p47phox in H₂O₂-induced hippocampal neurons [12]. Therefore, we speculated that Rg1 treatment might inhibit NOX2-mediated neuronal oxidative stress and neuroinflammation in APP/PS1 AD mice.

Increasing evidence suggests that neuroinflammation is an underlying cause of many human diseases and plays a catalyst-like role in the development of AD [18, 19]. The inflammatory response is not only a

reaction to the disease process but also a contributor to the neuronal damage. The inflammasomes are multiprotein complexes in the cytoplasm that are responsible for the formation of pro-inflammatory molecules. The NLRP1, a critical component of the inflammasome, appears to be expressed ubiquitously, and a high level of NLRP1 is found in the brain, particularly in neurons [20, 21]. A recent study reported that the NLRP1 immunopositive neurons in AD brains were increased by 25 to 30 folds compared with non-AD brains [21]. Similarly, inhibiting the expression of NLRP1 could reduce the A β deposition in the brain of AD model mice [22]. Our previous study showed that the NOX2 and NLRP1 inflammasome were significantly activated in prolonged primary cultured hippocampal neurons [17]. However, it remains unclear whether Rg1 can improve cognitive function and ameliorate neuronal damage and A β deposition through inhibiting the NOX2-NLRP1 inflammasome activation in APP/PS1 AD mice.

The APP/PS1 model mice of AD exhibit remarkable elevation of A β production associated with certain behavioral abnormalities. It has been reported that the expression of NLRP1 inflammasome increased in the brain of APP/PS1 mice with increasing age [22]. In the present study, we hypothesized that Rg1 treatment could alleviate A β generation and deposition by inhibiting NOX2 and NLRP1 inflammasome activation in the APP/PS1 AD mice. Apocynin, a NOX inhibitor, can inhibit the activity of NADPH oxidase by interfering with the intracellular translocation of its two cytoplasmic components of p47phox and p67phox [23]. It has been reported that apocynin has beneficial antioxidant effects on neurological diseases [24, 25]. In this study, to confirm our hypothesis, we investigated the protective effects and mechanism of Rg1 and apocynin on the locomotor activity, cognitive ability, neuronal damage, A β deposition and NOX2-NLRP1 inflammasome activation in the APP/PS1 AD mice. Meanwhile, we further studied the effect of NLRP1 knockdown on cognitive impairment, neuronal damage and A β deposition in APP/PS1 AD mice.

Materials And Methods

Animals and treatment

The study was performed on APP/PS1 female mice and wild-type (WT) littermates. The APP/PS1 transgenic mice were purchased from the Model Animal Research Center of Nanjing University. Male APP/PS1 mice were used in the experiment, and age- and gender-matched WT littermates were used as controls. Mice were housed in standard laboratory conditions with free access to standard food and water. The mice were allowed to adapt to the laboratory conditions before testing. The experimental protocols were approved by the animal ethics committee of Anhui Medical University.

To study the protective effects of Rg1 on APP/PS1 mice, six months (M) old male WT and APP/PS1 mice were divided into 6 groups: WT normal control (n = 12), APP/PS1-6M control (n = 9), APP/PS1-9M model control (n = 12), APP/PS1 + apocynin (50 mg/kg, n = 12), APP/PS1 + Rg1 (5 mg/kg, n = 12), APP/PS1 + Rg1 (10 mg/kg, n = 12). Rg1 (Rg1 content > 98%; Chengdu Desite Biotechnology Co., China) and apocynin (Merck Millipore) were dissolved in distilled water and were administered intragastrically (ig, 0.1 ml/10 g

body weight) for 12 weeks. The WT-9M and the APP/PS1-9M received the same amount of water for 12 weeks.

To study the effect of NLRP1-siRNA on APP/PS1 mice, six months old male APP/PS1 mice were divided into 3 groups (n = 8): normal saline control, lentivirus (LV)-scramble negative control, LV-NLRP1-siRNA-treated group. The negative lentivirus vector sequence is 5-TTC TCC GAA CGT GTC ACG T-3, NLRP1-siRNA is 5-GCT CTT CTT CTT CTT CTA ACA-3 (1×10^7 TU/ml, Shanghai GenePharma Co., China), which we have reported to significantly decrease NLRP1 expression in our previous study [26]. NS, control lentivirus or NLRP1-siRNA lentivirus (2 μ l each) were stereotactically injected into the left cerebral lateral ventricles as described in the previous study (coordinates: 0.6mm posterior to bregma; lateral: -1.5 mm; dorsoventral: 1.7 mm) [27]. The mice were maintained for 12 weeks after the injection of the lentivirus. After the behavior test, the mice were decapitated and the brains were quickly removed for other experiments.

Open field test (OFT)

The OFT equipment (Shanghai Biotechnology Co., Ltd.) includes a video-tracked cage (60 × 60 × 50 cm) and ANY-maze behavior tracking software (Stoelting Co., Wood Dale, IL, USA). The cage was divided into nine squares by two horizontal lines and two vertical lines as described in a previous study [28]. For OFT, each mouse was placed in the cage for 2 minutes to adapt to the environment. Then the ANY-maze behavioral tracking software was used to record the motor activity for 3 min. The total moving distance (m), the mean movement speed (m/s), the number of lines crossed, and the number of stand up were calculated by the software to indicate the motor activity behavior. In the OFT, each group except the APP/PS1-6M group was evaluated motor activity changes at 6 months old and every 4 weeks thereafter.

Buried Food Test (BFT)

In recent years, it has been reported that olfactory dysfunction occurs in AD patients, even in an early stage of AD [29]. Therefore we further detected the change of olfactory dysfunction after the OFT experiment using the previously described method [30]. Briefly, at 24 h prior to testing, the mice were subjected to a food-restricted diet. All mice were habituated to the testing room for 1 h prior to testing, then the mouse was allowed to adapt to the cage for 5 min. A small piece of food was randomly placed 1 cm beneath the bedding in a corner of a clean testing cage with 3 cm of woodchip bedding. The mouse was then placed in the testing cage at a constant distance from the hidden food. The ANY-maze behavioral tracking software was used to record the latency (s) of the mouse to find the food. If the mouse failed to find the buried food within 5 min, the latency score was recorded as 300 s. Nine hours after the above experiment, the surface pellet test was performed using the same scheme but the pellet was placed on the surface to exclude possible motor disorders.

Morris water maze (MWM) test

MWM is an important method to detect learning and memory impairments [31]. The MWM test includes four consecutive daily training trials and the fifth day of exploration trials as described in our previous study [32]. The mean escape latency (MEL, s) of the four trials each day was recorded to indicate the learning performance. On the fifth day, the platform was removed from the pool, and each mouse performed a 60s swimming probe trial. The latency of first entry to the platform (LFP, s), the swimming time in the quadrant of the platform (STP, s), and the number of crossing the platform (NCP) were recorded to indicate the memory results.

Histological examination

After the MWM test, the mice were sacrificed and were perfused transcardially with 4% paraformaldehyde to fix the brains. Then paraffin-embedded brain tissues were sliced into 5 μm sections using a microtome (Leica, Nussloch, Germany). The sections were stained with hematoxylin and eosin (H&E) to examine the pathological changes in cortex and hippocampus by intelligent tissue slice imaging analysis system platform (Pannoremic MIDI, 3DHISTECH, Hungary). Besides, Nissl staining is often used to identify neuronal damage [33]. We further performed the Nissl staining to study the neuronal pathological alterations. The sections were treated with Nissl staining solution (Beyotime Biotechnology, China) for 10 min and were differentiated with 95% alcohol. Then the sections were observed and photographed by a light microscope (Olympus IX71, Japan). The density of the Nissl bodies was analyzed double-blindly from three random fields (400 $\times$) in the cortex and hippocampal CA1 and CA3 regions in each section by using the Image-Pro Plus 6.0 automatic analysis system to assess the amount of Nissl bodies.

Determination of ROS production

For analysis of brain ROS production, the dihydropyridine (DHE) fluorescence staining was performed to examine the ROS levels in the brain [34]. Briefly, DHE (Beyotime Biotechnology, Shanghai, China) was injected from the tail vein (100 μM , 0.1 ml/10g body weight) for 30 minutes. Then the brains were removed and embedded in OCT (Sakura, Torrance, CA) at $-20\text{ }^{\circ}\text{C}$. The brain was cut into 10 μm sections by using a freezing microtome (Leica CM3050, Germany). Then the sections were washed and stained with DAPI for 5 minutes and were photographed by using a fluorescence microscope (Olympus IX71). The exposure time was 900 ms for all sections. The mean fluorescence density was quantified double-blindly from three random fields (400 $\times$) in the cortex, hippocampal CA1 and CA3 in each section by using Image-Pro Plus 6.0 automatic analysis system to assess the ROS production.

Determination of senescence-associated β -Galactosidase (β -Gal) activity

The β -galactosidase (β -Gal) is frequently used as a marker of cellular senescence. The frozen brain sections (5 μm) were stained with a β -Gal kit (Beyotime Biotechnology, Shanghai, China) to detect the β -Gal activity. Briefly, the sections were incubated with the β -Gal staining solution overnight at $37\text{ }^{\circ}\text{C}$. Blue products were generated under the catalysis of β -Gal with X-Gal as the substrate. The results were viewed by using a microscope (Olympus IX72, Japan). The mean density of the blue cells was quantified double-

blindly from three random fields (400×) in the cortex, hippocampal CA1 and CA3 in each section using Image-Pro Plus 6.0 automatic analysis system to assess the β -Gal activity.

Immunofluorescence

The paraffin sections were used to perform immunofluorescence. Briefly, the sections were deparaffinized and hydrated. Then the sections were permeabilized with Triton X-100 (0.25%) for 30 min, followed by blocking with BSA (1%) for 60 min. Then the sections were incubated with primary antibodies overnight at 4 °C. The antibodies were rabbit polyclonal postsynaptic density 95 (PSD95) (1:100, Servicebio Technology, GB11277), rabbit polyclonal $A\beta_{1-42}$ (1:150, Bioss Technology, bs-0076R). The next day, the sections were incubated with secondary antibodies against mouse or rabbit which were conjugated to Rhodamine (1:200, ZSGB-BIO). Then the sections were mounted by using an anti-fade medium and examined with the fluorescence microscope (Olympus IX71, Japan). The fluorescence density was analyzed double-blindly from three random regions (400×) of the cortex and hippocampus regions by using Image-Pro Plus 6.0 automatic analysis system to indicate the changes of PSD95 and $A\beta_{1-42}$ expression.

Thioflavin-S Staining

Paraffin sections (n = 5) were deparaffinized with xylene and were hydrated by graded alcohol. Then the sections were immersed in 1% Thioflavin-S (HY-D0972, MedChemExpress USA) for 5 minutes, hydrated in 70% ethanol and washed twice with PBS (5 minutes each time). After staining with Hoechst 33258 for 10 minutes, the slides were sealed with an anti-fade medium. The intelligent tissue slice imaging and analysis system platform (Pannoremic MIDI, 3DHISTECH, Hungary) was used to scan the images and analyze the data. The QuantCenter of Pattern of the platform is used to analyze the amyloid deposition areas and the hippocampus and cortex areas in each section. The percentage of Thioflavin-S positive areas was counted to evaluate changes in amyloid deposits.

Immunoblot analysis

The hippocampus and cortex tissues were homogenized in RIPA lysis (Beyotime Biotechnology, Shanghai, China) with protease and phosphatase inhibitors. Total protein was extracted using a cryogenic tissue grinder (Shanghai Jingxin Industrial Development Co., Ltd) at 4 °C, 60 Hz for the 50s. The BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China) was used to determine protein concentration. The samples (n = 4 per group) were separated by SDS-PAGE (8%–12%) and transferred onto PVDF membranes (Millipore, Bedford, MA, USA). Afterward, membranes were blocked in 5% non-fat milk in Tween20 TBS (TBST) for 1 h at room temperature, followed with the primary antibodies (**Supplementary Table S1**) overnight at 4°C. The next day, membranes were washed and incubated with a horseradish peroxidase-conjugated secondary antibody (ZSGB-BIO, ZF-2301, 1:10,000) for 1 h at room temperature. After washing three times with TBST, the proteins were visualized by an enhanced chemiluminescent reagent (Amersham Biosciences, UK). The bands were obtained by using a Chemi Q4800 mini imaging system (Shanghai Bioshine Technology, Shanghai, China). The density of the

protein band was measured using Image J 6.0. The density of the target band was normalized to β -actin. The relative density of target protein over WT control was used to indicate the changes in target proteins.

Quantitative real-time PCR (q-PCR) analysis

For the q-PCR analysis, total RNA was extracted from the hippocampus and cortex tissues ($n = 4$) with TRIzol reagent (Invitrogen Co., USA) according to the manufacturer's instructions as described previously [35]. The first-strand cDNA was synthesized from total RNA with PrimeScript™ Reverse Transcriptase (Takara Bio, RR037A) according to the manufacturer's protocol. Quantitative real-time PCR analyses for mRNAs of NLRP1, ASC, Caspase-1, IL-1 β , NOX2, p22phox, p47phox, APP, BACE, NCSTN, and β -actin were performed with SYBR®Premix Ex Taq™II RT-PCR kits (Takara Bio, RR820A). The mRNA level of β -actin was used as an internal control. Primers are listed in **Supplementary Table S2**. PCR was performed at 95 °C for 30 s, followed by 40 cycles of amplification at 95 °C for 5 s, 60 °C for 30 s with Real-time PCR System (BIO-RAD, CFX96). The fluorescent signals were collected during the extension phase, CT values of the sample were calculated, and transcript levels were analyzed by $2^{-\Delta\Delta CT}$ method. The q-PCR was repeated three times.

Statistical analysis

All data are expressed as mean $\pm$ SD. Statistical analysis was performed using GraphPad Prism 6.0 software. Repeated measures analysis of variance (ANOVA) was used to analyze the results of OFT from 6-9 months and MWM training from D1-4, and other data were analyzed using one-way ANOVA. Student's t -test was used to compare the differences between groups. Statistical significance was defined as $P < 0.05$.

Results

Rg1 treatment ameliorates abnormal behaviors in APP/PS1 mice

In this study, the open field test (OFT) was used to observe the effects of Rg1 treatment on motor behavior in APP/PS1 mice. The results showed that there were no significant differences between the WT-6M and APP/PS1-6M groups before Rg1 and apocynin treatment. While compared with 6M group, there were a decreasing trend in the moving distance (m), the mean moving speed (m/s), the number of lines crossing and the stand up with the age from 6M to 9M in WT control group and there were significant decrease in 8M and 9M (Fig. 1A-D, $P < 0.05$ or $P < 0.01$). In the APP/PS1-9M model group, these parameters also showed a decreasing trend, but the difference has no significant (Fig. 1A-D, $P > 0.05$). And in the apocynin and Rg1 (5 and 10 mg/kg) groups, similar to WT control group, these parameters also had a significant decrease, especially in 8M and 9M old (Fig. 1A-D, $P < 0.05$ or $P < 0.01$). Meanwhile, the results in 9M showed that, compared with WT-9M control, the moving distance (m) and mean moving speed (m/s) were significantly increased in APP/PS1-9M group, and the Rg1 (10 mg/kg) could significantly decrease the moving distance and mean moving speed compared with APP/PS1-9M group (Fig. 1A and B, $P < 0.01$). The number of lines crossing and stand up also decreased in the Rg1-treated

groups but there were no statistical differences (Fig. 1C and D). The data suggested that, with the increase of age, the APP/PS1 mice showed significant motor hyperactivity, Rg1 treatment significantly decreased the motor hyperactivity in the APP/PS1 mice.

We further used the BFT to detect the effect of Rg1 treatment on the alteration of olfactory function in APP/PS1 mice. The buried pellet results showed that there were no differences in the latency to find the food between the WT-9M and APP/PS1-6M group mice, while the latency was significantly increased in the APP/PS1-9M group mice compared with WT-9M group mice. Rg1 and apocynin treatment could significantly decrease the latency to find the food (Fig. 1E, $P < 0.05$ or $P < 0.01$). Meanwhile, the surface pellet test showed similar results to the buried pellet test (Fig. 1F, $P < 0.01$), but the latency to find the food was significantly shortened in all groups mice, suggesting there was no motor dysfunction in the experimental animals. These results suggested that Rg1 treatment could significantly improve the olfactory dysfunction in APP/PS1 mice.

We also use the MWM test to detect the effect of Rg1 treatment on learning and memory impairments in the APP/PS1 mice. The results of training trials showed that the escape latency has no significant difference in the first day (D1) among these experimental groups. However, compared with the D1, the escape latency in WT-9M group was significantly reduced in the D3 and D4 (Fig. 1G, $P < 0.01$), the APP/PS1-6M group also had a decreasing trend and significantly reduced in D4 (Fig. 1G, $P < 0.05$), while the escape latency had no significant decrease in APP/PS1-9M group (Fig. 1G, $P > 0.05$). Similarly to WT-9M group, the escape latency in apocynin and Rg1 treatment groups also had a decreasing trend and significantly reduced in D4 (Fig. 1G, $P < 0.05$ or $P < 0.01$). Additionally, compared with the WT-9M group, the escape latency of APP/PS1-9M group was significantly increased in D2, D3 and D4 (Fig. 1G, $P < 0.05$ or $P < 0.01$). While compared with APP/PS1-9M group, treatment with apocynin and Rg1 (10 mg/kg) could significantly reduce the escape latency in D4 (Fig. 1G, $P < 0.05$ or $P < 0.01$). In the fifth day of probe test, the results showed that, compared with WT-9M or APP/PS1-6M group, the latency of first entry to the platform (LFP, s) was significantly increased (Fig. 1H, $P < 0.01$), the number of crossing the platform (NCP) (Fig. 1I, $P < 0.01$) and the swimming time in the quadrant of platform (STP, s) (Fig. 1J, $P < 0.05$ or $P < 0.01$) were significantly decreased in APP/PS1-9M mice. While compared with APP/PS1-9M group, treatment with apocynin and Rg1 (5 and 10 mg/kg) could significantly reduce the LFP, and increase the NCP and STP (Fig. 1H-J, $P < 0.05$ or $P < 0.01$). The results indicated that Rg1 treatment could significantly improve the learning and memory impairments in APP/PS1 mice.

Rg1 treatment alleviates neuronal damage in APP/PS1 mice

We observed the pathological changes using H&E and Nissl staining to evaluate the effect of Rg1 treatment on neuronal damage in APP/PS1 mice. The H&E results showed that there were no abnormal changes in the cortex and hippocampus in the WT-9M group mice. Compared with WT-9M group, there were a few neuronal pathological changes in the APP/PS1-6M group mice such as unclear nucleoli, karyopyknosis and amyloid plaque (as shown in red square), indicating that there were slight neuronal damages in cortex and hippocampus in the APP/PS1-6M mice. While in APP/PS1-9M mice, there were

obvious neuronal damage and amyloid plaque in the cortex and hippocampus. And compared with APP/PS1-9M mice, apocynin and Rg1 treatment could significantly reduce the neuronal damages and amyloid plaque in the cortex and hippocampus (Fig. 2A). The data suggested that Rg1 treatment could significantly alleviate the neuronal damage in the APP/PS1 mice.

Nissl staining results showed that there were abundant Nissl bodies in the cortex and hippocampal neurons in the WT-9M and APP/PS1-6M group mice. Compared with WT-9M group, the Nissl bodies were significantly decreased in hippocampus CA3 and cortex in APP/PS1-9M group mice (Fig. 2B and C, $P < 0.05$ or $P < 0.01$). While compared with APP/PS1-9M group, treatment with apocynin and Rg1 could significantly increase the Nissl bodies in the hippocampus CA3 and cortex in the APP/PS1 mice, especially in Rg1 10 mg/kg group (Fig. 2B and C, $P < 0.05$ or $P < 0.01$). The results indicated that Rg1 treatment had a protective effect on neuronal damage in APP/PS1 mice.

β -gal is also an important biomarker for neuronal aging. β -gal activity is often measured to evaluate the age-related neuronal damage [36]. In this study, we further examined the expression of β -gal by immunoblot. The results showed that there were few expression of β -gal in WT-9M and APP/PS1-6M group mice, while compared with WT-9M or APP/PS1-6M group, the expression of β -gal was significantly increased in APP/PS1-9M group (Fig. 2D and E, $P < 0.01$). And compared with APP/PS1-9M group, treatment with apocynin and Rg1 could significantly reduce the expression of β -gal in APP/PS1 mice (Fig. 2D and E, $P < 0.01$). Additionally, we also detected the β -gal activity by β -gal staining. The results are similar to the results of immunoblot (Fig. S1A-D). These results suggested that Rg1 treatment could alleviate aging-related neuronal damage in APP/PS1 mice.

Behavioral dysfunction usually accompanied by changes in synaptic structure and function [37]. To evaluate the effect of Rg1 treatment on changes of synaptic function, we further detected the expression of PSD95 by immunofluorescence and immunoblot. The immunofluorescence results showed that there was abundant PSD95 expression in the WT-9M group. Compared with WT-9M group, the expression of PSD95 decreased in APP/PS1-6M group, but there was no significant differences. While in APP/PS1-9M group, the expression of PSD95 was significantly reduced (Fig. 3A-D, $P < 0.01$). Compared with APP/PS1-9M, treatment with apocynin and Rg1 could significantly increase the expression of PSD95 in APP/PS1 mice (Fig. 3A-D, $P < 0.01$). The immunoblot results were similar to immunofluorescence in PSD95 expression (Fig. 3E and F, $P < 0.01$). The results indicated that Rg1 treatment might improve the synaptic dysfunction in APP/PS1 mice.

Rg1 treatment reduces A β deposition and Tau hyperphosphorylation in APP/PS1 mice

The amyloid- β deposition is closely related to the generation and progression of pathological characteristics in APP/PS1 mice [38]. In this study, we first detected the expressions of APP and its metabolizing enzymes by immunoblot. The results showed that, compared to WT-9M group, the expression levels of APP, CTF- β and BACE had no significant difference in APP/PS1-6M group mice, while in APP/PS1-9M group, they were significantly increased (Fig. 4A-D, $P < 0.01$). Compared with APP/PS1-9M group, treatment with apocynin and Rg1 could significantly decrease the expression levels of APP,

CTF- β and BACE in APP/PS1 mice (Fig. 4A-D, $P < 0.05$ or $P < 0.01$). The results also showed that the expression of NCSTN had no significant changes in all groups mice (Fig. 4A and E). We also further detected the mRNA levels of APP, BACE and NCSTN. The results showed that the mRNA levels of APP, BACE and NCSTN were similar to their protein expression (Fig. 4F-H, $P < 0.05$ or $P < 0.01$). These data suggested that Rg1 treatment could significantly decrease the expressions of APP and BACE, and do not affect NCSTN expression.

To further confirm the protective effect of Rg1 on APP/PS1 mice, we detected the A β deposition by Thioflavin-S staining and expressions of A β_{1-42} by immunofluorescence and immunoblot. The Thioflavin-S staining results showed that there were few A β deposition in the cortex and hippocampus in WT-9M group mice. And compared with the WT-9M group, the A β deposition had a slight increase in the APP/PS1-6M group mice, while in APP/PS1-9M group, the A β deposition was significantly increased in the cortex and hippocampus (Fig. 4I and J, $P < 0.01$). Compared with APP/PS1-9M group, treatment with apocynin and Rg1 could significantly decrease the A β deposition both in the cortex and hippocampus (Fig. 4I and J, $P < 0.05$ or $P < 0.01$). Additionally, to confirm the effect of Rg1 treatment on A β deposition in APP/PS1 mice, we further detect the expression of A β_{1-42} using immunofluorescence. The results were similar to the Thioflavin-S staining of A β deposition (Fig. S2A and B, $P < 0.01$). Meanwhile, the immunoblot results also showed a similar result in the expression of A β_{1-42} (Fig. 4K and L, $P < 0.01$). These results suggested that Rg1 could significantly inhibit A β deposition and reduce the formation of amyloid plaque.

In addition, we also observed the effect of Rg1 treatment on the expression levels of Tau and p-Tau. The results showed that there were no significant changes in the expression of Tau among all groups. While compared with WT-9M group, the expression level of p-Tau was significantly increased in APP/PS1-9M group mice (Fig. 4M and N, $P < 0.01$). Compared with APP/PS1-9M group, treatment with apocynin and Rg1 could significantly reduce the expression level of p-Tau (Fig. 4M and N, $P < 0.05$). The results suggested that Rg1 treatment might also alleviate the neurofilament tangle induced by hyperphosphorylation of Tau.

Rg1 treatment decreases ROS generation and NOX2 expression in APP/PS1 mice

The accumulation of ROS plays a vital role in promoting the pathogenesis of AD [2, 3]. We further detected the ROS production in the cortex and hippocampus in the APP/PS1 mice. The results showed that there was slight ROS production in the cortex and hippocampal CA1 and CA3 areas in WT-9M and APP/PS1-6M group mice, while the ROS production was significantly increased in the cortex and hippocampus in APP/PS1-9M group compared with the WT-9M group (Fig. 5A-D, $P < 0.01$). Compared with APP/PS1-9M, apocynin and Rg1 treatment could significantly reduce the ROS production in the cortex and hippocampal CA1, CA3 areas (Fig. 5A-D, $P < 0.01$).

We further detected the expressions of NADPH oxidase 2-related protein and mRNA of NOX2, p22phox and p47phox, which were closely involved in ROS generation in neurons. The immunoblot results showed

that, compared with WT-9M group, the expression levels of NOX2, p22phox and p47phox had no significant increase in the APP/PS1-6M group mice, while they were significantly increased in the APP/PS1-9M group mice (Fig. 5E-H, $P < 0.01$). Compared with the APP/PS1-9M group, apocynin and Rg1 treatment could significantly decrease the expression levels of NOX2, p22phox and p47phox (Fig. 5E-H, $P < 0.01$). Meanwhile, the q-PCR results showed that, similar to their proteins, the levels of NOX2, p22phox and p47phox mRNA were significantly increased in APP/PS1-9M group mice (Fig. 5I-K, $P < 0.01$), and were significantly decreased after treatment with apocynin and Rg1 in APP/PS1 mice (Fig. 5I-K, $P < 0.01$). These data suggested that Rg1 treatment might reduce ROS generation by inhibiting NOX2 expression in the APP/PS1 mice.

Rg1 treatment decreases the expression of NLRP1 inflammasome in APP/PS1 mice

It has been reported that NOX2 plays an important role in the activation of NLRP1 inflammasome [26]. Therefore, we further detected the expression of NLRP1 inflammasome-related proteins and mRNA of NLRP1, ASC, caspase-1 and IL-1 β , and detected the expression levels of NF- κ B and p-NF- κ B by immunoblot. The results showed that, compared with WT-9M group, there was no significant elevation in the protein and mRNA of NLRP1, ASC, caspase-1 and IL-1 β in APP/PS1-6M group mice, while in APP/PS1-9M group mice, the expression levels of protein and mRNA of NLRP1, ASC, caspase-1 and IL-1 β were significantly increased (Fig. 6A-I, $P < 0.05$ or $P < 0.01$). Compared with APP/PS1-9M group, treatment with apocynin and Rg1 could significantly reduce the expression levels of protein and mRNA of NLRP1, ASC, caspase-1 and IL-1 β (Fig. 6A-I, $P < 0.05$ or $P < 0.01$). The NF- κ B and p-NF- κ B results showed that there was no significant difference between the WT-9M and APP/PS1-6M groups. While compared with the WT-9M or APP/PS1-6M groups, the expression levels of NF- κ B and p-NF- κ B were significantly increased in the APP/PS1-9M group mice (Fig. 6J-L, $P < 0.01$). And compared with APP/PS1-9M group, treatment with apocynin and Rg1 significantly reduced the expression levels of NF- κ B and p-NF- κ B in APP/PS1 mice (Fig. 6J-L, $P < 0.05$ or $P < 0.01$). These results suggested that Rg1 treatment could alleviate the neuroinflammation by inhibiting NLRP1 inflammasome activation in the APP/PS1 mice.

NLRP1-siRNA treatment ameliorates abnormal behaviors in APP/PS1 mice

To confirm the effect of NLRP1 inflammasome activation on learning and memory impairments and A β deposition in APP/PS1 mice, we further observed the effect of LV-NLRP1-siRNA treatment for 12 weeks on cognitive dysfunction, neurodegeneration and A β deposition. We firstly examined the effect of NLRP1-siRNA treatment on the motor behavior in APP/PS1 mice by using the OFT. The results showed that there were no significant differences among the NS, LV-scramble and LV-NLRP1-siRNA groups at 6M before treatment. While compared with 6M group, there was a decreasing trend in the motor behavior from 6M to 9M in all groups mice, especially in the LV-NLRP1-siRNA group, the moving distance (m), the mean moving speed (m/s), the number of lines crossing and the stand up were significantly decreased at 9M (Fig. 7A-D, $P < 0.05$ or $P < 0.01$). Additionally, the results of 9M showed that, compared with NS control, LV-NLRP1-siRNA treatment could decrease the number of lines crossing and stand up behavior (Fig. 7C

and D, $P < 0.05$). These data suggested that NLRP1-siRNA treatment could decrease the hyperactivity behavior which increased in the APP/PS1 mice.

We further used the BFT to detect the effect of NLRP1-siRNA treatment on the alteration of olfactory function in APP/PS1 mice. The results showed that NLRP1-siRNA treatment had no significant effect on the latency to find the food in both the buried pellet and surface pellet test (Fig. 7E and F, $P > 0.05$). The results suggested that NLRP1-siRNA treatment did not affect the olfactory dysfunction in the APP/PS1 mice.

We also use the MWM test to detect the effect of NLRP1-siRNA treatment on learning and memory impairments in the APP/PS1 mice. The results of training trials showed that the escape latency has no significant difference in the first day (D1) and D2 among the NS, LV-scramble and LV-NLRP1-siRNA treatment groups. However, compared with NS control group, the escape latency was significantly reduced in the D3 and D4 in the NLRP1-siRNA treatment group (Fig. 7G, $P < 0.05$). On the fifth day of the probe test, the results showed that, compared with NS control group, NLRP1-siRNA treatment could significantly reduce the LFP, and increase the NCP and STP in APP/PS1 mice (Fig. 7H-J, $P < 0.05$). The results indicated that NLRP1-siRNA treatment significantly alleviates the learning and memory impairments in APP/PS1 mice.

NLRP1-siRNA treatment inhibits NLRP1 inflammasome activation in APP/PS1 mice

We firstly confirmed the transfected effect of lateral ventricles injection of lentivirus with GFP. The results showed that treatment with lentivirus for 12 weeks showed obvious expression of GFP in hippocampus CA1, CA3 and cortex, indicating successful lentivirus infection in cortex and hippocampus in APP/PS1 mice (Fig. S3). Meanwhile, we further detected the effect of NLRP1-siRNA treatment on the expression levels of NLRP1-related protein and mRNA. The results showed that there were no significant differences between the NS and LV-scramble groups. While compared with NS or LV-scramble treatment group, NLRP1-siRNA treatment for 12 weeks could significantly decrease the protein and mRNA levels of NLRP1, ASC, caspase-1 and IL-1 β which increased in APP/PS1 mice (Fig. 8A-I, $P < 0.01$). We also detected the expression levels of NF- κ B and p-NF- κ B. The results showed that, compared with NS or LV-scramble treatment group, NLRP1-siRNA treatment could significantly decrease the expression levels of NF- κ B and p-NF- κ B in APP/PS1 mice (Fig. 8J and K, $P < 0.01$). These results suggested that NLRP1 knockdown could significantly improve the abnormal behavior and ameliorate the neuroinflammation in APP/PS1 mice.

NLRP1-siRNA treatment alleviates neuronal damage in APP/PS1 mice

We further used the H&E and Nissl staining to observe the effect of NLRP1-siRNA treatment on neuronal damage in APP/PS1 mice. The H&E results showed that there was significant neuronal damage and amyloid plaque in the cortex and hippocampus in NS and LV-scramble control group (Fig. 9A). Compared with the NS control group, NLRP1-siRNA treatment could significantly alleviate the neuronal damage and amyloid plaque in the cortex and hippocampus (Fig. 9A). The Nissl staining results also showed that

NLRP1-siRNA treatment could significantly increase the Nissl bodies in the cortex and hippocampal CA3 regions compared with NS control group (Fig. 9B and C, $P < 0.05$). To confirm the protective effect of NLRP1-siRNA treatment on neuronal damage in APP/PS1 mice, we further detected the expression levels of β -Gal and PSD95 by immunoblot. The results showed that NLRP1-siRNA treatment could significantly decrease the expression of β -Gal which was significantly increased in NS- and LV-scramble-treated APP/PS1 mice (Fig. 9D and E, $P < 0.01$). And NLRP1-siRNA treatment significantly increased the expression of PSD95 compared with NS or LV-scramble control group (Fig. 9F and G, $P < 0.01$). These results indicated that NLRP1 knockdown could alleviate neuronal damage and synaptic dysfunction in APP/PS1 mice.

NLRP1-siRNA treatment reduces A β deposition in APP/PS1 mice

To confirm whether NLRP1 knockdown could ameliorate the A β generation and deposition, we further detected the protein and mRNA of APP and its metabolic enzymes by immunoblot and q-PCR. The immunoblot results showed that, compared with NS group, LV-scramble treatment had no effect on the APP, CTF- β , BACE and NCSTN expressions, while NLRP1-siRNA treatment could significantly decrease the APP, CTF- β and BACE expressions (Fig. 10A-D, $P < 0.01$), and had no effect on NCSTN expression (Fig. 9A and E). The q-PCR results also showed a significant decrease in APP and BACE mRNA expression in NLRP1-siRNA treatment group (Fig. 10F and G, $P < 0.01$), and did not affect NCSTN mRNA expression (Fig. 10H).

Additionally, we further detected the expression of A β_{1-42} by immunofluorescence and immunoblot. The results showed that, compared with the NS group, NLRP1-siRNA treatment significantly decreased the expression of A β_{1-42} in the cortex and hippocampus in APP/PS1 mice (Fig. 10I-L, $P < 0.05$ or $P < 0.01$). Meanwhile, we also detected the effect of NLRP1-siRNA treatment on Tau phosphorylation. The results showed that NLRP1-siRNA treatment could significantly decrease the level of p-TAU which significantly increased in the NS group (Fig. 10M and N, $P < 0.01$). These results suggested that NLRP1 knockdown could inhibit A β generation and deposition in APP/PS1 mice.

Discussion

Alzheimer's disease (AD) is characterized by progressive cognitive impairments, A β deposition, Tau hyperphosphorylation and neurodegeneration in cortex and hippocampus. At present, it is still not completely understood the pathogenesis of AD, but growing evidence has focused on ROS oxidative stress and neuroinflammation, which may be closely involved in the pathogenesis in AD. Currently, there were no fully effective medicines and methods for the treatment of AD. Ginsenoside Rg1 (Rg1) is a bioactive component in ginseng, which has been used to delay senescence since ancient times. Our previous studies suggested that Rg1 could ameliorate H₂O₂-induced hippocampal neuron damage in vitro [12]. The APP/PS1 transgenic mouse is the most commonly used AD model animal, which exhibits obvious elevation of A β deposition and behavioral abnormalities. In this study, we hypothesized that Rg1 treatment might improve learning and memory, alleviate A β deposition and neuronal damage by

inhibiting NOX2-NLRP1 inflammasome activation and reducing ROS oxidative stress and neuroinflammation in APP/PS1 mice. The present results showed that Rg1 treatment could significantly alleviate the learning and memory impairments and neuronal damage, reduce the expressions of NOX2 and NLRP1 inflammasome, inhibit the expressions of APP, BACE and A β ₁₋₄₂, and decrease A β deposition and Tau hyperphosphorylation in APP/PS1 mice. Meanwhile, the results also showed that apocynin (a NOX inhibitor) and NLRP1-siRNA treatment had similar effects to Rg1 in the APP/PS1 mice. Our results suggest that Rg1 treatment may be a potential therapeutic strategy for delaying the progression of AD.

The APP/PS1 transgenic mouse is the most commonly used AD model, which develops spatial memory impairment, increased A β deposition in the brain, synaptic loss, and neuronal loss similar to those features observed in AD [39, 40]. It has been reported that the amyloid plaque can occur in the neocortex at 6-8 weeks and significantly increase at 8 months old [41], the synaptic dysfunction appears at about 3 months old [42], and the cognitive disability starts to show at about 6 months old in the APP/PS1 mice [43]. Therefore, we observed the effect of Rg1 treatment on APP/PS1 mice from 6M to 9M in the present study. In this study, the behavior results indicated that the APP/PS1-6M mice showed an increase of hyperactivity in the open field test (OFT) which also reported in other studies [44] and slight impairments of learning and memory in Morris water maze (MWM) test. But the APP/PS1-9M mice showed significant hyperactivity and learning and memory impairments compared with WT-9M group. We speculated that excessive A β generation and deposition might excite brain inducing hyperactivity and neuronal damage in the APP/PS1 mice. A recent study suggests that hyperactivity is an anxiety symptom, which negatively moderates the effect of A β on cognitive decline in preclinical AD, resulting in a more rapid decline in several cognitive domains [45]. The present results showed that Rg1 treatment could significantly decrease the hyperactivity and alleviate learning and memory impairments, suggesting a protective effect of Rg1 on abnormal behavior in APP/PS1-9M mice. Additionally, it was found that olfactory dysfunction (OD) occurred in AD patients, even in those who were at an early stage of AD [46]. A clinical study also suggests that OD is prevalent in AD patients, which shows the overall declines of the olfactory threshold, discrimination and identification [29]. The present results of the buried pellet test showed that there was significant OD in the APP/PS1-9M group mice which the latency to find food in the buried pellet test was significantly increased. However, treatment with Rg1 significantly reduced the latency to find food in the buried pellet test, suggesting Rg1 improved the OD in APP/PS1-9M mice.

AD-induced behavioral dysfunction is thought to be related to the structural and functional impairments of specific brain regions [47]. The cortex and hippocampus are primarily vulnerable regions affected by A β [48]. In this study, we found that there were slight A β deposition and neuronal damage in the cortex and hippocampus in APP/PS1-6M mice, while in the APP/PS1-9M mice, there were significant neuronal damage and A β deposition, and the Nissl bodies were significantly decreased in the cortex and hippocampus. However, Rg1 treatment could significantly alleviate neuronal damage and A β deposition. To confirm the protective effect of Rg1 treatment on APP/PS1 mice, we further examined the expression of senescence-associated β -Gal, which is a critical biomarker of cell aging. The results showed that Rg1 treatment also significantly decreased the expression of β -Gal, which was significantly increased in

APP/PS1-9M mice. The PSD95 is crucial for synaptic plasticity and is widely accepted as a regulatory protein that can regulate synapse function [49]. It has been reported that PSD95 is significantly reduced in the cortex and hippocampus of AD patients and negatively correlated with the severity of cognitive deficits [50]. The present study indicated that the expression of PSD95 was significantly decreased in the cortex and hippocampus, and was significantly increased after Rg1 treatment for 12 weeks in APP/PS1-9M mice, suggesting that Rg1 treatment might reverse the synaptic dysfunction of AD mice. These data suggested that the changes in neuronal damage and A β deposition were along with behavioral disorders in APP/PS1 mice, and Rg1 treatment could significantly alleviate the A β deposition and neuronal damage in APP/PS1 mice.

Amyloid plaques are important pathological characteristics of AD. A β , the main component of amyloid plaques, was shown to play a crucial role in the development of AD pathologies [51]. Recent study reported that excessive A β generation and deposition could change cell metabolism and trigger Tau hyperphosphorylation, synaptic dysfunction, oxidative stress and neuroinflammation, further leading to progressive recognition dysfunction [1]. A β is produced by sequential cleavage of β - and γ -secretases at the C terminus of amyloid precursor protein (APP) [51]. The APP can be cleaved by α -, β - and γ -secretase into multiple peptides and fragments complex. APP can be cleaved by β / γ -secretase into pernicious A β peptide and SAPP β fragments [52]. And APP can also be cleaved by α / γ -secretase to produce neuroprotective fragments of SAPP α and a nonamyloidogenic peptide [53]. BACE (beta-site APP-cleaving enzyme) is regarded as possessing β -secretase activity. Elevating the levels of BACE accelerates cleavage of APP into CTF- β . NCSTN is one of proteins that is regarded as possessing γ -secretase activity, which further cleaves CTF- β into A β . In the present study, the results showed that Rg1 treatment had no significant effect on NCSTN expression, but could significantly decrease the expression levels of APP, BACE and CTF- β , which were significantly increased in APP/PS1-9M mice. Meanwhile, we also detected the expression of A β ₁₋₄₂, a pernicious peptide, and the levels of amyloid plaque in the cortex and hippocampus. The results showed that Rg1 treatment significantly decreased the expression of A β ₁₋₄₂ and the levels of amyloid plaque in APP/PS1 mice. Additionally, Rg1 treatment significantly decreased the levels of p-Tau and senescence-associated β -Gal that were also significantly increased in the APP/PS1-9M mice. These results suggest that Rg1 may not only decrease APP expression but also inhibit the β -secretase pathway in APP processing to hinder pernicious A β formation.

Although the A β cascade is the most important pathogenesis of AD, there are still no clinical effective drugs acting on A β at present, suggesting other pathogenesis may be involved in AD. Growing evidence suggests that ROS oxidative stress and neuroinflammation play important roles in the progression of AD [19, 54]. Excessive ROS accumulation may even be one of the first pathogenic events during the progression of AD [55]. The NADPH oxidase (NOX) is an important enzyme that is responsible for ROS generation in many neurodegenerative diseases [56]. The NOX complex is currently the only enzyme family known to produce ROS as its sole function and comprises several isoforms such as NOX1-5. NOX2 is mainly expressed in neurons and composed of the membrane heterodimers of gp91phox (NOX2) and p22phox, and cytosolic subunits of p47phox, p67phox, p40phox and RAC [57]. NOX2-mediated

oxidative stress is closely involved in the progression of AD [58]. Neuroinflammation is considered to be a reaction and contributor to the neuronal injury and also plays an important role in the pathogenesis of AD [59]. Inflammasome plays a critical role in regulating the inflammatory response and contributes to the pathogenesis of various neurologic diseases [60]. The inflammasome is large intracellular multiprotein complexes, which contain a member of the NOD-like receptor (NLR) family, such as NLRP1-3 and IPAF, by which they are defined. The NLR protein recruits the inflammasome adaptor protein ASC, which in turn interacts with procaspase-1 leading to its activation, resulting in the maturation of the proinflammatory cytokines IL-1 β and IL-18. NLRP1 is the first reported inflammasome and is expressed extensively in many tissues. High-level of NLRP1 was found to be expressed in the central nervous system, especially in neurons [61]. Inhibition of NLRP1 expression could ameliorate age-related cognitive deficits [22]. However, it is still unclear whether activation of NOX2-NLRP1 inflammasome is involved in A β generation and deposition in the progression of AD.

Growing evidence suggests that Rg1 exerts significant antioxidant, anti-inflammatory and protective effects in neurological disease models in vivo and in vitro studies [62, 63]. Our previous study demonstrated that Rg1 treatment could reduce oxidative stress and neuroinflammation and delay neuronal senescence in hippocampal neurons in vitro [17]. The present results showed that Rg1 treatment significantly decreased the levels of ROS, IL-1 β in cortex and hippocampus. The further results demonstrated that Rg1 could significantly decrease the NOX2-related protein and mRNA levels of NOX2, p22phox and p47phox, and NLRP1 inflammasome-related protein and mRNA levels of NLRP1, ASC, caspase-1 and IL-1 β . Meanwhile, Rg1 treatment significantly decreased the level of p-NF- κ B in the APP/PS1 mice. These data suggested that the protective effects of Rg1 on APP/PS1 mice were closely related to inhibition of the NOX2-NLRP1 inflammasome pathway.

To further understand the molecular mechanisms underlying Rg1 actions, we examined the effects of apocynin and NLRP1-siRNA treatment on APP/PS1 mice. Apocynin is considered as an inhibitor of NOX to inhibit the generation of ROS. It has been reported that apocynin inhibits the NOX2 expression and ROS generation which increased by ischemia/reperfusion and significantly reduces the expressions of the NLRP3 inflammasome, IL-1 β and IL-18 in a mouse model of stroke [56]. NLRP1-siRNA has commonly used to down-regulate the expression of NLRP1 inflammasome. Our previous study indicated that NLRP1-siRNA (408) significantly reduced expressions of NLRP1 inflammasome and improve learning and memory impairments in dexamethasone-induced mice [26]. The present study showed that both apocynin and NLRP1-siRNA treatment could significantly alleviate the learning and memory impairments, synaptic dysfunction and neuronal damage in the APP/PS1 mice. The further results showed that apocynin and NLRP1-siRNA treatment could significantly decrease the expressions of APP, CTF- β , BACE and A β 1-42, and increase PSD95 expression in the APP/PS1 mice. These data suggest that both apocynin and NLRP1-siRNA treatment have protective effects on APP/PS1 AD mice. Furthermore, the results also showed that apocynin treatment could significantly decrease ROS generation and down-regulate the expression of NOX2-NLRP1 inflammasome in APP/PS1 mice. And NLRP1-siRNA treatment also significantly down-regulated the expression of NLRP1 inflammasome in the APP/PS1 mice. These data suggested that activation of NOX2-NLRP1 inflammasome was closely involved in the progression of

AD and down-regulation of NOX2-NLRP1 pathway significantly protected against cognitive dysfunction, A β deposition and neuronal damage in the APP/PS1 AD mice.

Conclusions

In summary, the present study suggested that Rg1 treatment could alleviate learning and memory impairment, synaptic dysfunction and neuronal damage and reduced A β generation and deposition in APP/PS1 mice. Meanwhile, we further found that Rg1 could significantly decrease the levels of ROS production and IL-1 β , and significantly inhibited the NOX2-NLRP1 inflammasome pathway in the brain cortex and hippocampus in APP/PS1 mice. These findings provided support that Rg1 could improve neuronal damage and delay the progression of AD, the mechanisms may be related to reducing neuroinflammation due to inhibition of NOX2-NLRP1 inflammasome activation. However, this study only provided a basic experiment for Rg1 in delaying the progression of AD in vivo, and the precise mechanisms of Rg1 on the regulation of the NOX2-NLRP1 pathway warrant further investigation.

Abbreviations

Rg1: Ginsenoside Rg1; NSAIDs: non-steroidal anti-inflammatory drugs; AD: Alzheimer's disease; A β : Amyloid beta; APP: amyloid precursor protein; BACE: beta-site APP-cleaving enzyme; p-Tau: phosphorylated Tau; β -Gal: β -Galactosidase; ROS: reactive oxygen species; NOXs: NADPH oxidases; NOX2: NADPH oxidase 2; NLRP1: nucleotide-binding oligomerization domain (NOD)-like receptor protein 1; DHE: dihydropyridine; PSD95: postsynaptic density 95; OD: olfactory dysfunction; OFT: open field test; MWM: Morris water maze; BFT: Buried Food Test; LFP: latency of first entry to the platform; NCP: number of crossing the platform; STP: swimming time in the quadrant of platform

Declarations

Acknowledgments

The authors thank Zhirui Fang in the Department of Pharmacology, Dake Huang and Bao Li in the Synthetic Laboratory of Basic Medicine College for their technical assistance.

Funding

This study was supported by the National Natural Science Foundation of China (81671384, 81970630).

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Weizu Li and Weiping Li designed the experiments. Han Zhang, Yong Su, Zhenghao Sun and Ming Chen experimented and analyzed experimental data. Han Zhang was a major contributor in writing the manuscript. Weizu Li wrote and refined the article. Yuli Han, Yan Li, and Xianan Dong participated in the behavioral experiments. Shixin Ding and Zhirui Fang participated in the immunofluorescence and immunoblot experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures were performed based on the guidelines and regulations approved by the Animal Care and Use Committee of Anhui Medical University (LLSC20160183).

Consent for publication

Not applicable.

Competing interests

None declared.

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Figures

Figure 1

Ginsenoside Rg1 treatment ameliorated abnormal behaviors in APP/PS1 mice. OFT: (A) The total moving distance (m); (B) The mean moving speed (m/s); (C) The number of stand up; (D) The number of lines crossing. BFT: (E) The latency of finding food in buried pellet test (s); (F) The latency of finding food in surface pellet test (s). MWM: (G) The mean escape latency (MEL, s) in the orientation navigation test; (H) The latency of first entry to the platform (LFP, s) in the probe test; (I) The number of crossing the platform

(NCP); (J) The swimming time in the quadrant of platform (STP, s). Results are expressed as mean $\pm$ SD. APP/PS1-6M group, n = 9; orther groups, n = 12. *P < 0.05, **P < 0.01 vs WT-9M group; #P < 0.05, ##P < 0.01 vs APP/PS1-6M group; Δ P < 0.05, $\Delta\Delta$ P < 0.01 vs APP/PS1-9M group; &P < 0.05, &&P < 0.01 vs D1 of 6M group in OFT and orientation navigation test of MWM.

Figure 2

Ginsenoside Rg1 treatment alleviated neuronal damage in cortex and hippocampus in APP/PS1 mice. (A) The results of H&E staining (Hippocampus 100 $\times$, the bar = 100 μ m; Cortex 400 $\times$, the bar = 20 μ m, n = 5). The red boxes indicate neuronal damage caused by A β deposition. (B) The results of Nissl staining in the cortex, hippocampus CA1 and CA3 (400 $\times$, the bar = 20 μ m); (C) The mean density of Nissl bodies in the cortex, hippocampus CA1 and CA3 (n = 5). (D) The bands of β -Gal and β -actin (Western blot); (E) The relative expression of β -Gal over WT-9M (n = 4). Results are expressed as mean $\pm$ SD. *P < 0.05, **P < 0.01 vs WT-9M group; #P < 0.05, ##P < 0.01 vs APP/PS1-6M group; Δ P < 0.05, $\Delta\Delta$ P < 0.01 vs APP/PS1-9M group.

Figure 3

Ginsenoside Rg1 treatment increased the expression of PSD95 in APP/PS1 mice. (A) The expression of PSD95 in the cortex, hippocampus CA1 and CA3 (immunofluorescence, 400×, the bar = 20 μm. Rg1 (5 mg/kg) group, n = 3, the other groups, n = 4); (B) The mean density of PSD95 in cortex; (C) The mean density of PSD95 in hippocampus CA1; (D) The mean density of PSD95 in the hippocampus CA3; (E) The bands of PSD95 and β-actin (Western blot); (F) The relative expression of PSD95 over WT-9M (n = 4).

Results are expressed as mean ± SD. *P < 0.05, **P < 0.01 vs WT-9M group; #P < 0.05, ##P < 0.01 vs APP/PS1-6M group; ΔP < 0.05, ΔΔP < 0.01 vs APP/PS1-9M group.

Figure 4

Ginsenoside Rg1 treatment reduced Aβ deposition in APP/PS1 mice. (A) The bands of APP, CTF-β, BACE, NCSTN and β-actin (Western blot); (B) The relative expression of APP over WT-9M; (C) The relative expression of CTF-β over WT-9M; (D) The relative expression of BACE over WT-9M; (E) The relative

expression of NCSTN over WT-9M; (F) The relative mRNA expression of APP (q-PCR); (G) The relative mRNA expression of BACE (q-PCR); (H) The relative mRNA expression of NCSTN (q-PCR); (I) The A β deposition in cortex and hippocampus (Thioflavin-S staining, 20 $\times$, the bar = 500 μ m, yellow arrows indicate A β deposition); (J) The ratio of A β plaque load (%) in cortex and hippocampus; (K) The bands of A β 1-42 and β -actin (Western blot); (L) The relative expression of A β 1-42 over WT-9M. (M) The bands of TAU and p-TAU (Western blot); (N) The relative expression of p-TAU/TAU over WT-9M. Results are expressed as mean $\pm$ SD, Thioflavin-S staining, n = 5; other experiments, n = 4. *P < 0.05, **P < 0.01 vs WT-9M group; #P < 0.05, ##P < 0.01 vs APP/PS1-6M group; Δ P < 0.05, $\Delta\Delta$ P < 0.01 vs APP/PS1-9M group.

Figure 5

Ginsenoside Rg1 treatment decreased the level of ROS and the expressions of NADPH oxidase 2 in APP/PS1 mice. (A) The ROS production in the cortex, hippocampus CA1 and CA3 (DHE fluorescence staining, 400×, the bar = 50 μm); (B) The mean density of ROS production in the cortex; (C) The mean density of ROS production in the hippocampus CA1; (D) The mean density of ROS production in the hippocampus CA3. (E) The bands of NOX2, p22phox, p47phox and β-actin (Western blot); (F) The relative

expression of NOX2 over WT-9M; (G) The relative expression of p22phox over WT-9M; (H) The relative expression of p47phox over WT-9M; (I) The relative mRNA expression of NOX2 (q-PCR); (J) The relative mRNA expression of p22phox (q-PCR); (K) The relative mRNA expression of p47phox (q-PCR). Results are expressed as mean $\pm$ SD, n = 4. *P < 0.05, **P < 0.01 vs WT-9M group; #P < 0.05, ##P < 0.01 vs APP/PS1-6M group; Δ P < 0.05, $\Delta\Delta$ P < 0.01 vs APP/PS1-9M group.

Figure 6

Ginsenoside Rg1 treatment decreased the expressions of NLRP1 inflammasome in APP/PS1 mice. (A) The bands of NLRP1, ASC, caspase-1, IL-1 β and β -actin (Western blot); (B) The relative expression of NLRP1 over WT-9M; (C) The relative expression of ASC over WT-9M; (D) The relative expression of caspase-1 over WT-9M; (E) The relative expression of IL-1 β over WT-9M; (F) The relative mRNA expression of NLRP1 (q-PCR); (G) The relative mRNA expression of ASC (q-PCR); (H) The relative mRNA expression of caspase-1 (q-PCR); (I) The relative mRNA expression of IL-1 β (q-PCR); (J) The bands of NF- κ B, p-NF- κ B, and β -actin (Western blot); (K) The relative expression of NF- κ B over WT-9M; (L) The relative expression of p-NF- κ B over WT-9M. Results are expressed as mean $\pm$ SD, n = 4. *P < 0.05, **P < 0.01 vs WT-9M group; #P < 0.05, ##P < 0.01 vs APP/PS1-6M group; Δ P < 0.05, $\Delta\Delta$ P < 0.01 vs APP/PS1-9M group.

Figure 7

NLRP1-siRNA treatment ameliorated abnormal behaviors in APP/PS1 mice. OFT: (A) The total moving distance (m); (B) The mean moving speed (m/s); (C) The number of stand up; (D) The number of lines crossing; BFT: (E) The latency of finding food in buried pellet test (s); (F) The latency of finding food in surface pellet test (s); MWM: (G) The mean escape latency (MEL, s) in the orientation navigation test; (H) The latency of first entry to the platform (LFP, s) in the probe test; (I) The number of crossing the platform

(NCP); (J) The swimming time in the quadrant of platform (STP, s). Results are expressed as mean $\pm$ SD, $n = 8$. * $P < 0.05$, ** $P < 0.01$ vs APP/PS1-9M group; & $P < 0.05$, && $P < 0.01$ vs D1 of 6M group in OFT and orientation navigation experiment of MWM.

Figure 8

NLRP1-siRNA treatment decreased the expressions of NLRP1 inflammasome in APP/PS1 mice. (A) The bands of NLRP1, ASC, Caspase-1, IL-1 β , and β -actin (Western blot); (C) The relative expression of NLRP1 over NS; (D) The relative expression of ASC over NS; (E) The relative expression of caspase-1 over NS; (F) The relative expression of IL-1 β over NS; (G) The relative mRNA expression of NLRP1 (q-PCR); (H) The relative mRNA expression of ASC (q-PCR); (I) The relative mRNA expression of caspase-1 (q-PCR); (J) The relative mRNA expression of IL-1 β (q-PCR); (K) The bands of NF- κ B, p-NF- κ B, and β -actin (Western blot); (L) The relative expression of NF- κ B over NS; (M) The relative expression of p-NF- κ B over NS. Results are expressed as mean $\pm$ SD, $n = 4$. * $P < 0.05$, ** $P < 0.01$ vs APP/PS1-NS control group.

Figure 9

NLRP1-siRNA treatment alleviated neuronal damage in cortex and hippocampus in APP/PS1 mice. (A) The results of H&E staining (Hippocampus 100×, the bar = 100 μm; Cortex 400×, the bar = 20 μm). The red boxes indicate neuronal damage caused by Aβ deposition. (B) The results of Nissl staining in the cortex, hippocampus CA1 and CA3 (400×, the bar = 20 μm); (C) The mean density of Nissl bodies in the cortex, hippocampus CA1 and CA3. (D) The bands of β-Gal and β-actin 9 (Western blot); (E) The relative

expression of β -Gal over WT-9M. (F) The bands of PSD95 and β -actin (Western blot); (G) The relative expression of PSD95 over NS. Results are expressed as mean $\pm$ SD, n = 4. *P < 0.05, **P < 0.01 vs APP/PS1-NS control group.

Figure 10

NLRP1-siRNA treatment reduced A β deposition in APP/PS1 mice. (A) The bands of APP, CTF- β , BACE, NCSTN and β -actin (Western blot); (B) The relative expression of APP over NS; (C) The relative expression

of CTF- β over NS; (D) The relative expression of BACE over NS; (E) The relative expression of NCSTN over NS; (F) The relative mRNA expression of APP (q-PCR); (G) The relative mRNA expression of BACE (q-PCR); (H) The relative mRNA expression of NCSTN (q-PCR) ; (I) The expression of A β 1-42 in cortex and hippocampus (immunofluorescence, 20 $\times$, the bar = 500 μ m, yellow arrows indicate A β deposition); (J) The ratio of A β 1-42 plaque load (%) in cortex and hippocampus; (K) The bands of A β 1-42 and β -actin (Western blot); (L) The relative expression of A β 1-42 over NS; (M) The bands of TAU and p-TAU (Western blot); (N) The relative expression of p-TAU/TAU over NS. Results are expressed as mean $\pm$ SD, n = 4. *P < 0.05, **P < 0.01 vs APP/PS1-NS control group.

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