Current Medical Science
DOI https://doi.org/10.1007/s11596-019-2019-1
39(2):196-203,2019

Notoginseng Saponin Rg1 Prevents Cognitive Impairment through Modulating APP Processing in $A\beta_{1-42}$-injected Rats*

Shang-zhi LIU[1], Wei CHENG[1], Jia-wei SHAO[1], Yun-fan GU[1], Yi-yi ZHU[1], Qi-jing DONG[1], Si-yu BAI[1], Ping WANG[2#], Li LIN[1#]
[1]*Cell Molecular Biology Laboratory of Basic Medical College, Hubei University of Chinese Medicine, Wuhan 430065, China*
[2]*Hubei Research Institute of Geriatrics, Collaborative Innovation Center of Hubei Province, Hubei University of Chinese Medicine, Wuhan 430065, China*

Summary: With the intensification of the aging process of the world, Alzheimer's disease (AD), which is the main type of senile dementia, has become a primary problem in the present society. Lots of strategies have been used to prevent and treat AD in animal models and clinical trials, but most of them ended in failure. Panax notoginseng saponins (PNS) contain a variety of monomer compositions which have been separated and identified. Among of the monomer compositions, notoginseng saponin Rg1 (Rg1) accounts for 20% of the cultivation of panax notoginseng roots. And now PNS have been reported to be widely used to treat cardio-cerebrovascular diseases and have neuroprotective effects to restrain the β-amyloid peptide (Aβ)25–35-mediated apoptosis. Moreover, it is reported that PNS could accelerate the growth of nerve cells, increase the length of axons and promote synaptic plasticity. Whether Rg1 can ameliorate the cognitive impairment and the underlying mechanism has not been elucidated. To study the preventive effect of Rg1 on cognitive impairment and the possible mechanism, we established the cognitive impairment model in rats through $A\beta_{1-42}$ (2.6 μg/μL, 5 μL) injection and then treated the rats with Rg1 (25, 50 and 100 mg/kg) administered intragastrically for 4 weeks. We observed that $A\beta_{1-42}$ could induce spatial learning and memory deficits in rats. Simultaneously, $A\beta_{1-42}$ injection also resulted in the reduced neuron number in cornuammonis 1 (CA1) and dentate gyrus (DG) of hippocampus, as well as the increased level of hyperphosphorylated β-amyloid precursor protein (APP) at Thr668 site with up-regulation of β-APP cleaving enzyme 1 (BACE1) and presenilin 1 (PS1) and down-regulation of a disintegrin and metalloprotease domain-containing protein 10 (ADAM10) and insulin-degrading enzyme (IDE). Administration of Rg1 effectively rescued the cognitive impairment and neuronal loss, and inhibited the β-secretase processing of APP through reducing APP-Thr668 phosphorylation and BACE1/PS1 expression, and increasing the expression of ADAM10 and IDE. We concluded that Rg1 might have neuroprotective effects and could promote learning and memory ability, which might be a viable candidate in AD therapy probably through reducing the generation of Aβ and increasing the degradation of Aβ.
Key words: notoginseng saponin Rg1; Alzheimer's disease; spatial learning and memory deficits; β-amyloid peptide; secretase; degrading enzyme

The senile dementia is a neurodegenerative disease with aging as the main cause, and Alzheimer's disease (AD) is the main type of senile dementia with pathological manifestations of the senile plaques formed by the aggregation of extracellular β-amyloid peptide (Aβ) in brain tissue, and the neurofibrillary tangles (NFTs) and neuronal loss formed by the aggregation of intracellular hyperphosphorylation of tau protein[1, 2]. Pathological hallmarks occur in the frontal and temporal lobes of cerebral cortex, the hippocampus and basal forebrain. It is always considered that formation of Aβ plaques leads to NFTs and causes neuronal cell death and cognitive impairment[3, 4]. Furthermore, many studies indicated the occurrence of AD was closely related to inflammation,

Shang-zhi LIU, E-mail: 1337033157@qq.com
#Corresponding authors, Li LIN, E-mail: linli@hbtcm.edu.cn; Ping WANG, E-mail: pwang54@aliyun.com
*This project was supported in parts by grants from the National Natural Science Foundation of China (No. 81673856, and No. 81573865), Postdoctoral Science Foundation of China (No. 2016M592319, and No. 2017T100542) and National Innovation and Entrepreneurship Training Program for College Students (No. 201810507019).

cholinergic nerve injury, oxidative stress and so on[5]. At present, drugs that were researched and developed according to the above theories to prevent and cure AD mostly ended in failure. Meanwhile, the situation of those drugs that are used in clinical treatment or research is not optimistic and it may be related to the complex pathogenesis of AD. Traditional Chinese medicine (TCM) devotes particular care to the overall view, adjusting the body balance of Yin and Yang. The efficacy of TCM in prevention and treatment of many chronic diseases, including dementia is gradually accepted by doctors and patients[6].

Notoginseng is one of the Chinese medicines which is used to stop bleeding, promote blood circulation by removing blood stasis and tonify the blood, in line with the theory of TCM treatment of AD by activating blood and resolving stasis. Notoginseng contains notoginsenosides, polysaccharides, dencichines, flavonoids, amino acids and other substances, and notoginsenosides are the main active constituent of notoginseng. The total arasaponin contains various monomer components that have been isolated and identified. Among of the monomer compositions, notoginseng saponin Rg1 (Rg1) is believed to be about 20% of notoginseng roots. It has been reported that notoginseng can enhance the memory and cognitive function, depress the free radical toxicity impairment, restrain the apoptosis regulating genes, and has a certain therapeutic effect on AD[7, 8]. However, the involved thorough molecular mechanisms need further study.

In the present study, we established AD-like model in rats by lateral ventricle injection of $A\beta_{1-42}$. We found that $A\beta_{1-42}$ injection could lead to spatial learning and memory deficits in rats in the Morris water maze (MWM) with the decreased number of nerve cells in hippocampal cornuammonis 1 (CA1) and dentate gyrus (DG) regions, as well as the increased level of $A\beta_{1-42}$ and the decreased ratio of $A\beta_{1-40}/\beta_{1-42}$. We also found that $A\beta_{1-42}$ could induce the up-regulation of phosphorylated β-amyloid precursor protein (APP) at Thr668 (pAPP668), β-APP cleaving enzyme 1 (BACE1) and presenilin 1 (PS1), as well as down-regulation of a disintegrin and metalloprotease domain-containing protein 10 (ADAM10) and insulin-degrading enzyme (IDE). Simultaneously, treatment with Rg1 rescued the $A\beta_{1-42}$-induced spatial learning and memory impairments and the associated variations.

1 MATERIALS AND METHODS

1.1 Antibodies and Chemicals

The antibodies used in this study are listed in table 1. $A\beta_{1-42}$ polypeptide (Sigma, USA) was dissolved in dimethysulfoxide (DMSO) and added to the saline solution to form a solution of 2.6 μg/μL. Then it was incubated at 37°C for one week and placed at 4°C for standby. Bicinchoninic acid (BCA) protein detection kit was purchased from Pierce Chemical Company (USA).

Table 1 A list of antibodies and their epitopes on the molecule of protein used in this study

Antibody	Specificity	Type	Dilution	Source
APP	Mouse	Mono-	1:1000 for WB	Millipore (USA)
pAPP668	Rabbit	Poly-	1:1000 for WB	Cell Signaling (USA)
ADMA10	Rabbit	Poly-	1:300 for WB	Cell Signaling (USA)
BACE1	Rabbit	Poly-	1:500 for WB	Cell Signaling (USA)
PS1	Rabbit	Poly-	1:500 for WB	Cell Signaling (USA)
IDE	Rabbit	Poly-	1:1000 for WB	Cell Signaling (USA)
GAPDH	Mouse	Mono-	1:1000 for WB	Abcam (UK)

Mono-, monoclonal; Poly-, polyclonal; WB, Western blotting

1.2 Drug Administration

Three-month-old (weighing 230±20 g) male Sprague-Dawley (SD) rats were supplied by the Centers for Disease Control in Hubei Province (China). All experimental procedures were approved by the Animal Care and Use Committee at the Hubei University of Chinese Medicine and were performed in obeying National Institutes of Health guidelines on the ethical use of animals. Rats were fed in house of a 12 h light:12 h dark (L/D) cycle with the light on from 8:00 am to 8:00 pm and were treated just as that was described in fig. 1A.

The rats were placed in a Jiangwan-Ⅱ stereotaxic instrument (Jiangwan Medical Instrument Co., China) after they were anesthetized with 6% chloral hydrate (400 mg/kg)[9]. The hair between the ears and eyes was shaved off and the scalp was incised (8.0–10.0 mm). The skull was cleaned to expose the anterior fontanelle adequately and then the hole (diameter 1.0 mm) was made with dental drill for the infusion. For the lateral ventricular infusion, the coordinate of AP-0.8 mm, L-1.5 mm, V-4.0 mm (from bregma and dura, flat skull) was selected according to the stereotaxic atlas of Fanklin and Paxinos. A sterilized needle connected to a Hamilton syringe was used to inject 5 μL $A\beta_{1-42}$ into the lateral ventricle. Equal volume of DMSO with

physiological saline was infused as vehicle controls. The needle was retained for 5 min after $A\beta_{1-42}$, or DMSO with physiological saline injection and then slowly retrieved and the pinhole was covered with medical gelatin sponge to prevent the injection and cerebrospinal fluid to drain out. Then penicillin was daubed and the skin was stitched with surgical suture after the incision was disinfected.

1.3 Behavioral Test

Spatial learning and memory were measured by Morris water maze (MWM) test[10]. The MWM apparatus was the same as previously described by our team[11]. The water temperature was maintained at 24±2°C, and the appropriate nontoxic black ink was added into the pool to make the water opaque which could contrast sharply with the rat's white skin to capture the rat's trajectory easily. The swimming pathways and the latencies of the rats to find the hidden platform were recorded each day, meanwhile, the times of crossing the platform on the 7th day were also recorded by the apparatus attached to a computer.

1.4 Nissl Staining

The brains of rats were fixed for 1 h by transcardial perfusion with 4% paraformaldehyde and then successive coronal sections of 20 μm were made with freezing microtome (CM1860S, Leica, Germany). The picked brain slices were pasted on the glass slide disposed by gelatin in PBS liquid, dustproof atmospheric drying for 24 h. And then the appropriate 1% toluidine blue was dropped on the brain slices, keeping on horizontal position for 5–10 min, then 95% alcohol was used to differentiate and the brain slice was observed under a microscope at the same time until the background was clean and the Nissl body was clear. If the dyeing was light, the 1% toluidine blue could be dropped on the brain slice again to redye and 95% alcohol was used to differentiate again. Then the brain slices were dehydrated with 100% alcohol for 5 min × 2, and transparently handled with xylene for 5 min × 2 and sealed with neutral gummi. Finally, the brain slices were observed under a microscope and the images were collected to analyze.

1.5 ELISA

Hippocampus tissue levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were measured by ELISA. Protein levels of hippocampus homogenate samples were normalized by BCA protein assay. $A\beta_{1-40}$ and $A\beta_{1-42}$ were quantified in these samples using the $A\beta_{1-40}$ and $A\beta_{1-42}$ ELISA kits according to the manufacturer's instructions. A standard curve was prepared from standard substance in duplicate. The levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the hippocampus were determined from the standard curve expressed as pg/mg tissue protein.

1.6 Western Blotting

Western blotting was carried out as described previously by our team[10]. Briefly, the proteins of the extracts were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane after the proteins' concentration was detected with BCA kit (Pierce, USA). The membranes were probed with primary antibody at 4°C overnight and then were incubated with anti-mouse or anti-rabbit IgG conjugated to horseradish peroxidase for 1 h at room temperature. Then the grey scale images were obtained and analyzed with odyssey system.

1.7 Statistical Analysis

Data were expressed as mean±standard deviation (SD) and analyzed using SPSS 16.0 statistical software (SPSS Inc., USA). The data of the MWM test were analyzed with a two-way analysis of variance (ANOVA). The other data were evaluated by a one-way ANOVA procedure followed by LSD's post hoc Bonferroni tests, which were used to determine the different means among groups. The level of significance was set at $P<0.05$.

2 RESULTS

2.1 Rg1 Rescues the Spatial Learning and Memory Deficits through Increasing the Number of Neurons in CA1 and DG of Hippocampus in Rats Induced by $A\beta_{1-42}$

To produce an *in vivo* AD-like spatial learning and memory deficits model, we infused $A\beta_{1-42}$ (2.6 μg/μL, 5 μL) into the lateral ventricle of rats and measured the spatial learning and memory abilities by the MWM test. To study the effects of Rg1 on $A\beta_{1-42}$-induced spatial learning and memory deficits, the AD-like model rats were treated with different concentrations of Rg1 with intragastric administration (25, 50 and 100 mg/kg, serving as Rg1-L, Rg1-M and Rg1-H groups respectively). To measure the effects of Rg1 on cognitive impairment, we trained the rats for 6 consecutive days to allow them remembering the hidden platform in water maze and the hippocampus-dependent spatial memory was measured by removing the platform on the 7th day (fig. 1B and 1C). The results showed that the escape latency of finding the hidden platform decreased to varying degrees in experimental rats of all the groups during 6 days of training and testing. From day 3 onward, the model group rats had longer escape latency to find the hidden platform (fig. 1B). The rats in the groups treated with Rg1 found the platform within 15 s by a direct searching strategy on the 6th day, and in the model group, the latency was increased to about 40 s (fig. 1B). Simultaneously, there was significant difference in the times of crossing the platform between the control group and the model group ($P<0.05$, 0.01; fig. 1C). Rg1 rescued $A\beta_{1-42}$-induced learning and memory deficits shown by the significantly decreased escape latency as well as the increased times of crossing the platform (Rg1 of

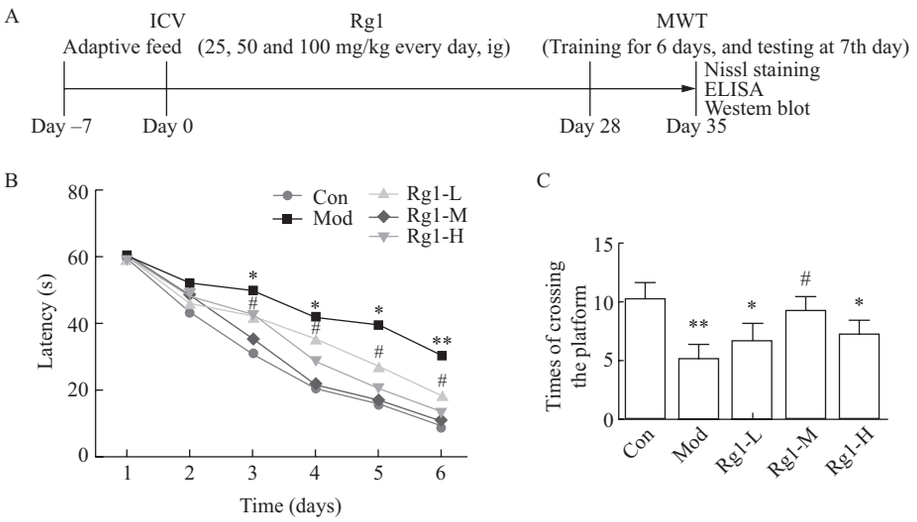

Fig. 1 $A\beta_{1\text{-}42}$ induces learning and memory deficits that can be reversed by Rg1

A: the schema chart of the experimental design shown as the pattern diagram. B: Sixty male SD rats were randomly divided into five groups and injected with 2.6 μg/μL $A\beta_{1\text{-}42}$ (5 μL) through the lateral ventricle except the control group (Con) which was injected with the same volume and concentration of DMSO. The three treated groups (Rg1-L, Rg1-M and Rg1-H) were treated with low (25 mg/kg), middle (50 mg/kg) and high (100 mg/kg) concentrations of Rg1 respectively by intragastric administration (ig) and the Con group and model group (Mod) were treated with the same volume of 0.9% sodium chloride for 4 weeks. Then the MWM test was used to assess the learning and memory abilities of these rats. $A\beta_{1\text{-}42}$ could induce learning and memory deficits shown by the increased latency, which could be reversed by Rg1. C: Furthermore, the medium dose of Rg1 could more significantly increase the times of crossing the platform than Rg-L and Rg-H groups.

ICV, intracerebroventricular injection; MWM, Morris water maze. The data were expressed as means±SD (n=12). $^{*}P$<0.05, $^{**}P$<0.01 *vs.* Con; $^{\#}P$<0.05 *vs.* Mod

middle concentration group, Rg1-M) in the MWM test. These data suggest that $A\beta_{1\text{-}42}$ can induce acquisition and retention of memory deficits of rats, which can be improved by Rg1 especially in the middle dose of Rg1.

The cortex and hippocampus are closely related to the learning and memory ability. To explore the mechanisms underlying the $A\beta_{1\text{-}42}$-induced spatial learning and memory deficits, we measured the neurons of cortex and hippocampus by Nissl staining. The results indicated there were neuronal loss in CA1 and DG subsets of hippocampus of the model group as compared with the control group. The neuronal loss was not seen in cortex and CA3 subsets, suggesting that $A\beta_{1\text{-}42}$ induced cell death in CA1 and DG subsets. Rg1 could reverse the cell death in CA1 and DG subsets (fig. 2A–2C).

2.2 Rg1 Decreases $A\beta_{1\text{-}42}$ and Increases $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ Ratio in Hippocampus

Another characteristic pathological change of AD is the formation of extracellular senile plaques (SP) which is mainly composed of Aβ. Studies indicated that $A\beta_{1\text{-}42}$ increased at the early stage of AD and decreased at the late stage. The expression of $A\beta_{1\text{-}42}$ was significantly increased after $A\beta_{1\text{-}42}$ injection (fig. 3B), and there was no significant difference in the $A\beta_{1\text{-}40}$ expression before and after $A\beta_{1\text{-}42}$ injection (fig. 3A). Nevertheless, the ratio of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ decreased obviously after $A\beta_{1\text{-}42}$ injection. Rg1 could reverse the increase in $A\beta_{1\text{-}42}$ and the decreased ratio of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ (fig. 3A–3C).

2.3 Rg1 Treatment Reduces Production, and Increases Degradation of Aβ-related α, β, γ Secretases and Degradation Enzyme

To further determine whether Rg1 reversed the learning and memory deficits induced by $A\beta_{1\text{-}42}$ through modulating the activities of secretase of Aβ, we measured the levels of total APP, pAPP668 and α-secretase (eg. ADAM10), β-secretase (eg. BACE1) and γ-secretase (eg. PS1) as well as the degradation enzyme of Aβ (eg. IDE) in the hippocampus region by Western blot. The results indicated that there was no significant difference in the level of total APP between any two groups. $A\beta_{1\text{-}42}$ could induce an increase in pAPP668, BACE1 and PS1. In addition, the results showed a decrease in ADAM10 and IDE in the hippocampus in the model group as compared with the control group. And Rg1 could reverse these phenomena described above (fig. 4A–4E).

3 DISCUSSION

AD is clinically characterized by progressive cognitive deterioration. With the intensification of the aging process of the society, the incidence of dementia is also increasing. AD is the most common type of dementia among older people which accounted for 50%–75% of

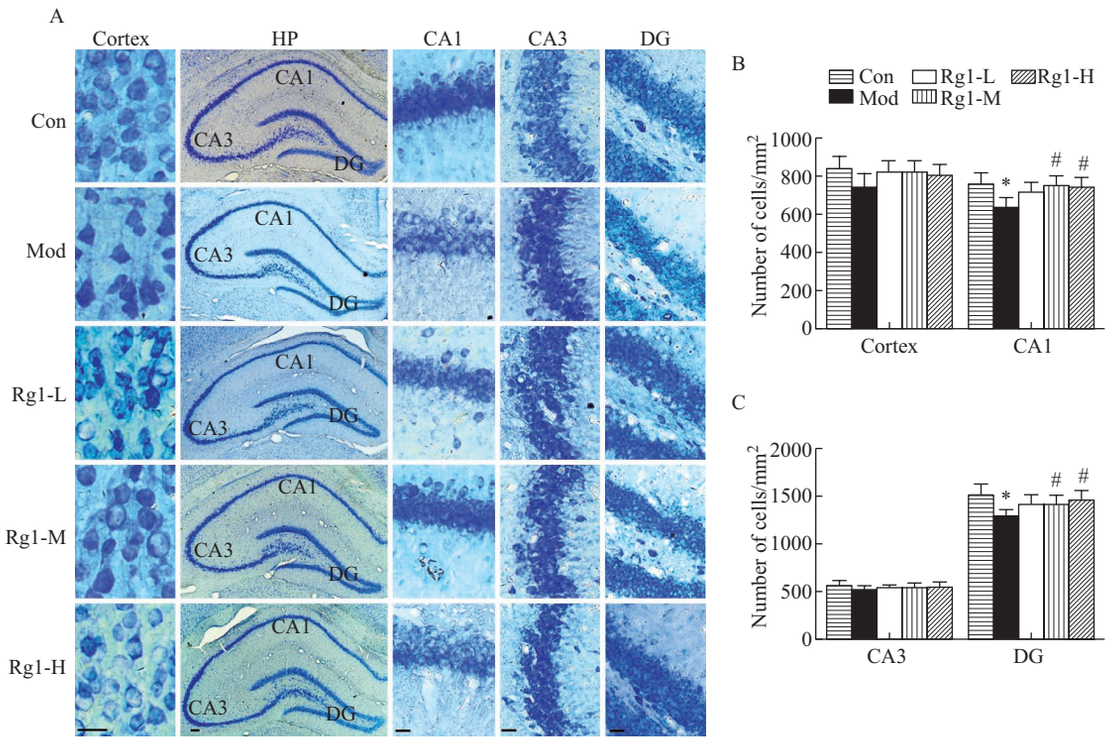

Fig. 2 $A\beta_{1\text{-}42}$ induces cell loss in hippocampal CA1 and DG subsets, which could be reversed by Rg1

A: the representative hippocampus Nissl staining after $A\beta_{1\text{-}42}$ injection and treatment with different doses of Rg1 for 4 weeks (scale bar=500 μm for HP; scale bar=100 μm for cortex and hippocampal CA1, CA3 and DG). B: The neuronal number in cortex and hippocampal CA1 region was analyzed. C: The neuronal number in hippocampal CA3 and DG regions was analyzed. Rg1 could reverse the neuronal loss in CA1 and DG regions of hippocampus induced by $A\beta_{1\text{-}42}$. The data were expressed as mean±SD (n=5). $^{*}P$<0.05 *vs.* Con, $^{\#}P$<0.05 *vs.* Mod

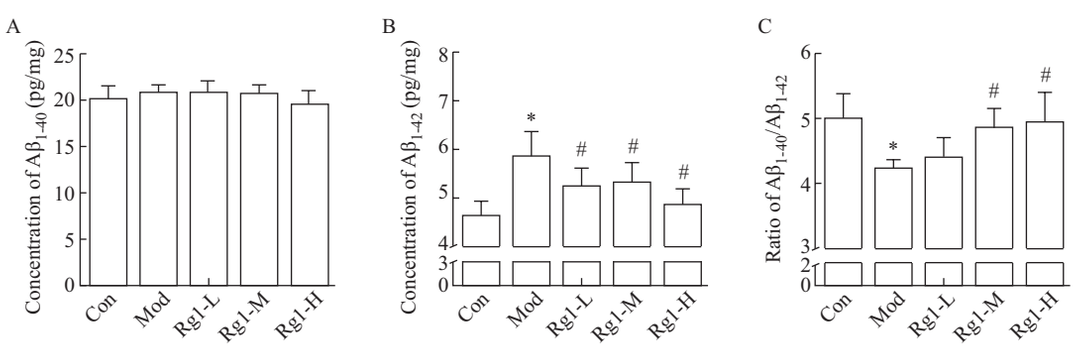

Fig. 3 Rg1 could rescue the alterations of $A\beta_{1\text{-}42}$ and the ratio of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ induced by $A\beta_{1\text{-}42}$ in rats

A, B and C: After behavior test, the brain extract from hippocampal regions was used to assess the alterations of $A\beta_{1\text{-}40}$ (A) and $A\beta_{1\text{-}42}$ (B) proteins by ELISA and ratio of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ (C). The level of $A\beta_{1\text{-}40}$ had no evident difference (A) but the level of $A\beta_{1\text{-}42}$ increased obviously after $A\beta_{1\text{-}42}$ injection, which could be rescued by Rg1 (B). At the same time, the ratio of $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ decreased significantly after $A\beta_{1\text{-}42}$ injection, which also could be reversed by mid- and high-dose of Rg1 (C). The data were expressed as mean±SD (n=4). $^{*}P$<0.05 *vs.* Con, $^{\#}P$<0.05 *vs.* Mod

all dementia cases. At present, the prevalence rate of AD is over 7.5% in people over 65 years old[12] and the number of AD patients in China is as high as 9 million now[13, 14]. The current approved drugs for treatment of AD based on neural protection hypothesis, such as donepezil, galantamine, rivastigmine, and memantine, aim to improve cognitive impairment through adjusting the excitatory neurotransmitter pathways, but their efficacy is limited. One of the current hot spots of AD researches is BACE1 inhibitors and Aβ vaccine. Groping for natural active ingredients from medicinal herbs to treat AD has attracted worldwide researchers' attention to Chinese medicine. At present, galantamine and huperzine A which were extracted from traditional Chinese herbs have been developed and used in clinics to treat mild to moderate type of AD[15, 16].

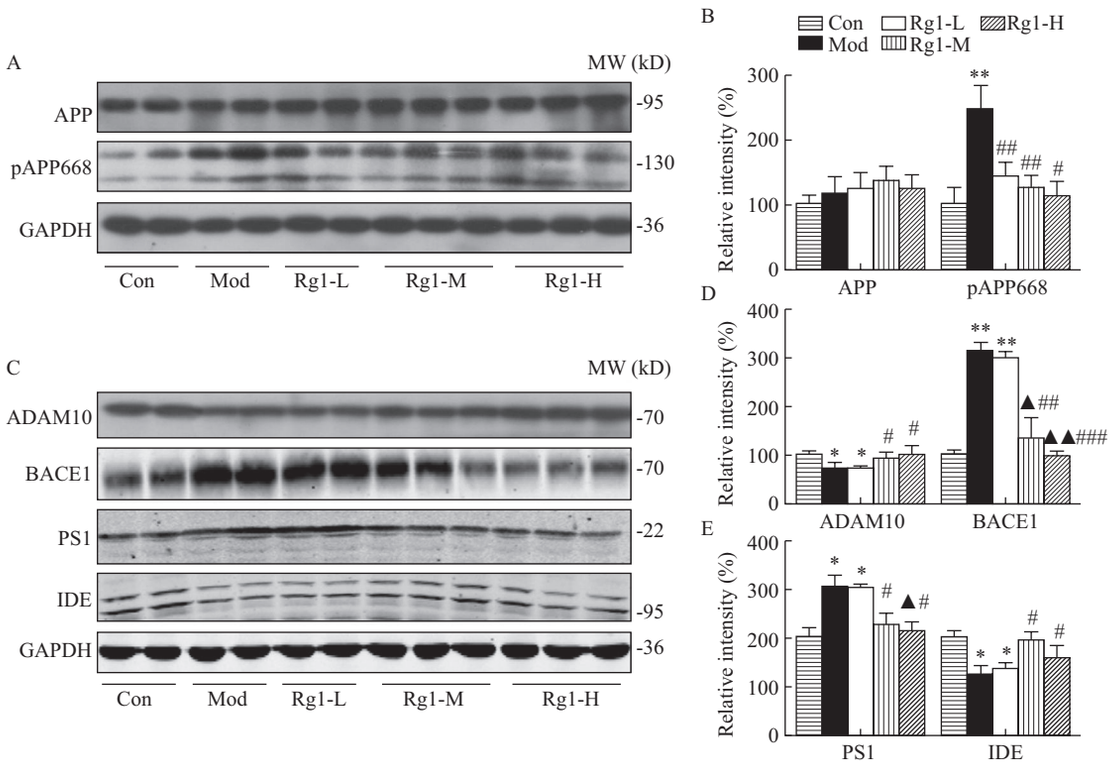

Fig. 4 $A\beta_{1\text{-}42}$-induced learning and memory deficits possibly by modulating the phosphorylated APP at Thr668 (pAPP668) and the relevant secretases of Aβ, and the reversal effects of Rg1

The levels of pAPP668, normalized against total APP and GAPDH, were increased (A) and quantitatively analyzed after $A\beta_{1\text{-}42}$ injection (B), and decreased after treatment with Rg1. The levels of ADAM10, BACE1, PS1 and IDE were detected by Western blotting and normalized against GAPDH (C) and quantitatively analyzed (D, E). Rg1 treatment reversed the increased BACE1 and PS1 and decreased ADAM10 and IDE induced by $A\beta_{1\text{-}42}$. The data were expressed as means±SD (n=3). $^{*}P$<0.05, $^{**}P$<0.01 *vs.* Con; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 *vs.* Mod; $^{\blacktriangle}P$<0.05, $^{\blacktriangle\blacktriangle}P$<0.01 *vs.* Rg1-L

Rg1, also called ginsenoside Rg1, is one of the main compounds in panax notoginseng and widely used in China for treatment of cardiocerebral vascular diseases. Recent studies suggested that panax notoginseng saponins (PNS) had the effects of lipid metabolism-regulating, anti-inflammatory, plaque-stabilizing, and anti-oxidation effects, and so PNS had the functions of anti-atherosclerosis and protecting the heart. It has been reported that Rg1 could regulate the ratio of Bcl-2 to Bax in the brain tissue of rats with cerebral ischemia injury as well as promote the recovery of nerve function[17]. Other studies demonstrated that Rg1 improved the learning and memory deficits in AD model mice induced by Aβ[18, 19]. *In vitro* studies suggested that Rg1 mitigated ROS injury on nerve cells and restrained apoptosis of rat nerve cells[20]. Our recent studies indicated Rg1 could improve $A\beta_{25\text{-}35}$-induced spatial learning and memory impairment of the AD model rats, and decrease the phosphorylation level of tau protein in hippocampus[21]. Whether the spatial memory deficit could be induced by lateral ventricular infusion of $A\beta_{1\text{–}42}$ and whether it could be ameliorated by Rg1? What are the probable mechanisms that are responsible for the production, and the increased degradation of Aβ?

In the present study, we further investigated the influences of Rg1 to the cognitive impairment model in rats induced by $A\beta_{1\text{–}42}$. Our data revealed that $A\beta_{1\text{–}42}$ could induce the spatial learning and memory disorders with characteristics of increasing the escape latency, decreasing the times of crossing the platform as compared with the control group rats. Meanwhile, Rg1 could reverse the phenomenon of spatial learning and memory deficits when the rats were treated with different concentrations of Rg1 (25, 50 and 100 mg/kg every day). Early studies identified Rg1 had neuroprotective effects through restraining the Aβ-mediated apoptosis. It has been confirmed that the occurrence of AD is closely related to the loss of neurons and neurofibrillary tangles, which are contributed to the aggregations of hyperphosphorylated Tau proteins and Aβ[22, 23]. And we speculated the spatial memory deficit induced by $A\beta_{1\text{-}42}$ was related to the loss of neurons and accumulations of Aβ. In the present study, we found that $A\beta_{1\text{-}42}$ could induce neuronal loss in the CA1 and DG regions of hippocampus, which could be ameliorated by Rg1. Simultaneously, we found that $A\beta_{1\text{–}42}$ treatment increased the level of $A\beta_{1\text{-}42}$ and decreased the ratio of

$A\beta_{1-40}$/$A\beta_{1-42}$. We observed that phosphorylated APP at Thr668 as well as BACE1 and PS1 increased after treatment with $A\beta_{1-42}$. At the same time, the level of ADAM10 and IDE decreased after $A\beta_{1-42}$ injection. After treatment with Rg1, these phenomena described above could be significantly improved. Under physiological conditions, APP can be cleaved by α-secretase within the Aβ domain to release soluble amino acid (sAPP) and APPα which can preclude generation of Aβ. Under pathological conditions, Aβ is first generated from APP through sequential cleavages by β-secretase and then by the γ-secretase complex. Studies have indicated that the activity of α-secretase is mediated by a series of membrane-bound proteases, which are members of the ADAM family including ADAM9, ADAM10 and ADAM17. While BACE1 belongs to β-secretase which is responsible for the generation of Aβ peptides. The activity of γ-secretase is associated with a multisubunit transmembrane complex containing at least four known subunits: presenilin (PS), presenilin enhancer 2 (Pen2), nicastrin (Nct), and anterior pharynx defective (Aph). PS, including two homologs, PS1 and PS2, is considered to be the core of the catalytic enzyme because it has been proved that active site-directed inhibitors specifically label PS and mutation of either of the two transmembrane aspartates results in loss of γ-secretase activity which was fixed by Aph.

It is speculated that ginsenosides can possess the ability of passing through BBB and has no non-toxicity. Some studies have shown that the phosphorylated APP at Thr668 site (pAPP668) is more easily transported to nerve endings and the level of pAPP668 in AD neurons increases, suggesting that phosphorylated APP at this site can regulate the production of Aβ. The present study indicated the level of pAPP668 was augmented after $A\beta_{1-42}$ injection, which could be reversed by Rg1. Some other studies showed that the increased α-secretase-mediated processing of APP could reduce the processing of APP being cleaved by β-secretase that could decrease the production of Aβ[24, 25]. Our present study indicated that Rg1 increased α-secretase level probably by aggrandizing the level of ADAM10, and prompted that augmenting level of α-secretase may be one of the mechanisms of Rg1 reducing Aβ production. It has been reported that BACE1 controls the first and rate-limiting step of Aβ generation from APP and thus it has been considered as one of the most promising potential therapeutic targets to treat AD[26, 27]. In fact, several BACE1 inhibitors are currently being studied in clinical trials. Our present work indicated that Rg1 decreased β-secretase level probably by downregulating the level of BACE1, which suggested Rg1 may be a latency BACE1 inhibitor to treat AD. It has also been reported that γ-secretase was a multisubunit aspartyl protease which could cleave APP and has been certified to be a high tractable target for AD treatment[28]. Our study indicated that Rg1 decreased γ-secretase level probably by downregulating the level of PS1, which suggested Rg1 may be a latency PS1 inhibitor to treat AD. IDE was considered as the degrading enzyme of Aβ and our data showed that Rg1 aggrandized the level of IDE, which prompted Rg1 could increase the degrading of Aβ for controlling AD.

Taken together, this study demonstrates that Rg1 has key functions in ameliorating spatial learning and memory deficits induced by $A\beta_{1-42}$ in rats with the neuroprotective effects by reducing the generation and increasing the degradation of Aβ through augmenting the level of α-secretase and degrading enzyme and debasing the level of β-secretase and γ-secretase. Thus, it may suggest that Rg1 has neuroprotective effect through reducing the generation and increasing the degradation of Aβ involved in $A\beta_{1-42}$-induced toxicity in our experimental model.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

REFERENCES

1 Huang HC, Jiang ZF. Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease. J Alzheimers Dis, 2009,16(1):15-27

2 Grundke-Iqbal I, Iqbal K, Quinlan M, *et al*. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem, 1986,261(13):6084-6089

3 Bernardo A, Mccord M, Troen AM, *et al*. Impaired spatial memory in APP-overexpressing mice on a homocysteinemia-inducing diet. Neurobiol Aging, 2007,28(8):1195-1205

4 Long Z, Zeng Q, Wang K, *et al*. Gender difference in valproic acid-induced neuroprotective effects on APP/PS1 double transgenic mice modeling Alzheimer's disease. Acta Biochim Biophys Sin, 2016,48(10):930-938

5 Grimaldi A, Brighi C, Peruzzi G, *et al*. Inflammation, neurodegeneration and protein aggregation in the retina as ocular biomarkers for Alzheimer's disease in the 3xTg-AD mouse model. Cell Death Dis, 2018,9(6):685

6 Ong WY, Wu YJ, Farooqui T, *et al*. Qi Fu Yin-a Ming Dynasty Prescription for the Treatment of Dementia. Mol Neurobiol, 2018,55(9):7389-7400

7 Chen B, Qin MC, Huang JL, *et al*. Preliminary establishment of integration of Alzheimer's disease and blood stasis syndrome tree shrew model and evaluation of intervention of Panax notoginseng saponins. Zhongguo Zhong Yao Za Zhi (Chinese), 2017,42(6):1175-1182

8 Choi RC, Zhu JT, Leung KW, *et al*. A flavonol glycoside, isolated from roots of Panax notoginseng, reduces amyloid-beta-induced neurotoxicity in cultured neurons: signaling transduction and drug development for Alzheimer's disease. J Alzheimers Dis, 2010,19(3):795-811

9 Zhu LQ, Wang SH, Ling ZQ, *et al*. Inhibition of

melatonin biosynthesis activates protein kinase a and induces Alzheimer-like tau hyperphosphorylation in rats. Chin Med Sci J, 2005,20(2):83-87
10 Yin YY, Liu H, Cong XB, *et al*. Acetyl-L-carnitine attenuates okadaic acid induced tau hyperphosphorylation and spatial memory impairment in rats. J Alzheimers Dis, 2010,19(2): 735-746
11 Liu SJ, Zhang AH, Li HL, *et al*. Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. J Neurochem, 2003,87(6):1333-1344
12 Sisodia SS. Alzheimer's disease: perspectives for the new millennium. J Clin Invest, 1999,104(9):1169-1170
13 Gao J, Inagaki Y, Li X, *et al*. Research progress on natural products from traditional Chinese medicine in treatment of Alzheimer's disease. Drug Discov Ther, 2013,7(2):46-57
14 Liu Q, Wang SC, Ding K. Research advances in the treatment of Alzheimer's disease with polysaccharides from traditional Chinese medicine. Chin J Nat Med, 2017,15(9):641-652
15 Wang R, Yan H, Tang XC. Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. Acta Pharmacol Sin, 2006,27(1):1-26
16 Takata K, Kitamura Y, Saeki M, *et al*. Galantamine-induced amyloid-{beta} clearance mediated via stimulation of microglial nicotinic acetylcholine receptors. J Biol Chem, 2010,285(51):40 180-40 191
17 Zhu MX, Ran B, Feng ZQ, *et al*. Effects of Rb1 and Rg1 on the expression of Bcl-2, Bax in apoptosis of HK-2 cells induced by the serum of kidney ischemia/reperfusion. Zhongguo Ying Yong Sheng Li Xue Za Zhi (Chinese), 2009,25(4):496-499
18 Wang Y, Kan H, Yin Y, *et al*. Protective effects of ginsenoside Rg1 on chronic restraint stress induced learning and memory impairments in male mice. Pharmacol Biochem Behav, 2014,120: 73-81
19 Kezhu W, Pan X, Cong L, *et al*. Effects of Ginsenoside Rg1 on Learning and Memory in a Reward-directed Instrumental Conditioning Task in Chronic Restraint Stressed Rats. Phytother Res, 2017,31(1):81-89
20 Yang M, Chuan Y, Guo C, *et al*. Panax notoginseng Root Cell Death Caused by the Autotoxic Ginsenoside Rg1 Is Due to Over-Accumulation of ROS, as Revealed by Transcriptomic and Cellular Approaches. Front Plant Sci, 2018,9(2):264
21 Li MZ, Wu WH, Wu ZP, *et al*. Panax notoginseng saponins Rg1 improving the spatial cognitive ability and decreasing the phosphorylation level of tau protein in AD model rats. Acta Med Univ Sci Technol Huazhong (Chinese), 2017,46(3):248-252
22 Alonso AD, Grundke-Iqbal I, Barra HS, *et al*. Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. Proc Natl Acad Sci USA, 1997,94(1):298-303
23 Takashima A. Hyperphosphorylated tau is a cause of neuronal dysfunction in tauopathy. J Alzheimers Dis, 2008,14(4):371-375
24 Allinson TM, Parkin ET, Turner AJ, *et al*. ADAMs family members as amyloid precursor protein alpha-secretases. J Neurosci Res, 2003,74(3):342-352
25 Yan R, Bienkowski MJ, Shuck ME, *et al*. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature, 1999,402(6761):533-537
26 Yan R, Fan Q, Zhou J, *et al*. Inhibiting BACE1 to reverse synaptic dysfunctions in Alzheimer’s disease. Neurosci Biobehav Rev, 2016,65(6):326-340
27 Coimbra J, Marques D, Baptista SJ, *et al*. Highlights in BACE1 Inhibitors for Alzheimer's Disease Treatment. Front Chem, 2018,6(5):178
28 Song H, Chang YJ, Moon M, *et al*. Inhibition of glutaminyl cyclase ameliorates amyloid pathology in an animal model of Alzheimer's disease via the modulation of gamma-secretase activity. J Alzheimers Dis, 2015,43(3):797-807

(Received Nov. 15, 2018; revised Feb. 27, 2019)