ORIGINAL ARTICLE

Simvastatin Exerts Antiamnesic Effect in A β ₂₅₋₃₅-Injected MiceWen-Hong Zhi,^{1,2} Yan-Ying Zeng,² Zi-Hong Lu,¹ Wei-Jun Qu,¹ Wei-Xian Chen,² Lei Chen¹ & Ling Chen¹¹ Department of Physiology, Nanjing Medical University, Nanjing, China² Department of Geriatric Neurology, Jiangsu Province Hospital, Nanjing, China

Keywords

Neuroprotection; Simvastatin; Synaptic plasticity; $\alpha 7$ nicotinic acetylcholine receptor; β -amyloid peptide.

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Received 3 July 2013; revision 13 September 2013; accepted 19 September 2013

doi: 10.1111/cns.12190

SUMMARY

Aim and Methods: Simvastatin (SV) is reported to improve cognition and slow the progression of Alzheimer's disease (AD). This study explored the mechanisms underlying the antiamnesic effect of SV in AD using behavior tests, histological examination, western blot analysis, and electrophysiological recording technique in AD model mice created by intracerebroventricular injection (i.c.v.) of A β ₂₅₋₃₅. **Results:** Chronic administration of SV (40 mg/kg/day) for 11 days after A β ₂₅₋₃₅-injection ameliorated the impairment of acquisition performance and probe trail test in Morris water maze task and alternation behavior in Y maze task in A β ₂₅₋₃₅-mice. A β ₂₅₋₃₅-induced apoptosis of hippocampal CA1 pyramidal cells and A β ₂₅₋₃₅-impaired high-frequency stimulation (HFS)-dependent long-term potentiation (LTP) induction in hippocampal Schaffer collaterale-CA1 synapse were rescued by SV-treatment. SV prevented A β ₂₅₋₃₅-inhibited protein kinase B (Akt) and extracellular signal-related kinase-2 (ERK2) phosphorylation, which was sensitive to $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) antagonist MLA. SV-induced neuroprotection was attenuated by MLA or phosphatidylinositol-3-kinase (PI3K) antagonist LY294002. SV-rescued LTP induction was blocked by $\alpha 7$ nAChR, PI3K or MAPK/ERK kinase (MEK) antagonist. Finally, the antiamnesia of SV in A β ₂₅₋₃₅-mice was attenuated by blockage of SV-induced neuroprotection or SV-rescued LTP induction. **Conclusion:** The antiamnesia of SV in A β ₂₅₋₃₅-mice depends on its neuroprotection and synaptic plasticity improvement.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and a common cause of dementia in the elderly. The main neuropathological characteristics in AD include deposition of β -amyloid peptide (A β), plaque formation, neurofibrillary tangles and impairment of cholinergic system [1]. Besides this, a cerebrovascular pathology is also a clinical landmark of the disease [2]. AD patients display reduced cerebral blood flow at rest and lessened perfusion to activated areas. Statins, a direct inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, are widely used for the treatment of hypercholesterolemia [3]. Epidemiological evidence shows a good relevance between statins therapy and a low incidence of AD, particularly in patients with cerebrovascular disease [4]. Clinical trials have proved that simvastatin (SV) can improve cognitive function of mild to moderate AD and slow the progression of AD [5,6]. Tong et al. report that SV, if initiated early in the disease process, is fully effective against both vascular and memory deficits of adult but not aged AD transgenic mice [7]. The antiamnesia of SV is independent of cholesterol-lowering effect or changes in A β levels or plaque load [4,7,8], and the underlying mechanism still remains unclear.

The $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) are expressed in hippocampus where they regulate neurotransmitter release, activate intracellular signaling pathways, and influence

downstream processes involved in learning and memory [9]. It has been proved that $\alpha 7$ nAChR may be one of the most likely targets of A β -neurotoxicity [9,10]. Lovastatin can up-regulate $\alpha 7$ nAChR in cultured neurons, which is independent of changes in cholesterol levels [11]. Furthermore, blockade of $\alpha 7$ nAChR by cholinesterase inhibitors (ChEIs) is prevented by application of mevastatin and lovastatin [12]. These reports indicate that statins may modulate $\alpha 7$ nAChR at the receptor level.

A β containing the 11 amino acids (25–35) (A β ₂₅₋₃₅) retains the ability to self-aggregate and has been proved to be neurotoxic *in vitro* and *in vivo* [13,14]. An animal model of AD-type amnesia created by intracerebroventricular injection (i.c.v.) of A β ₂₅₋₃₅ has been widely used to analyze the behavioral and neuropathological consequences of A β -neurotoxicity *in vivo* [10,14–16]. Previous studies show that injection (i.c.v.) of A β ₂₅₋₃₅ in mice causes impairment in learning and memory ability and neuronal loss within 1 or 2 weeks after administration, which can be reversed by cholinergic agonist [10,14,15]. Here, we studied the effects of a commonly used statin drug, simvastatin which can easily pass through blood–brain barrier [17], on A β ₂₅₋₃₅-induced deficits in spatial memory behavioral, hippocampal morphological, electrophysiological, and biochemical changes, and the role of $\alpha 7$ nAChR in SV-action. The study demonstrated that the administration of SV in A β ₂₅₋₃₅-mice exerted a potent antiamnesic effect, which was mediated through SV-induced neuroprotection and SV-improved

synaptic plasticity. The results also showed that α 7nAChR-mediated phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt) and extracellular signal-related kinase (ERK) signaling pathways were involved in the beneficial effect of SV in AD.

Materials and methods

Experimental Animals

Male mice (ICR, Oiental Bio Service Inc., Nanjing, China), weighing 20–25 g at the beginning of the experiment, were used in the study. The animals were housed in a light controlled room under a 12-h light–dark cycle starting at 7:00 AM and kept at a temperature of 23°C. They received food and water *ad libitum*. Experiments were approved by the Nanjing Medical University Animal Care and Use Committee. Care of animals conformed to standards established by the National Institutes of Health and all efforts were made to minimize animal suffering and to reduce the number of animals used.

Preparation of an Animal Model of Alzheimer's Disease

"Aggregated" A β _{25–35} (Sigma Chemical Co., St. Louis, MO, USA) was obtained by incubating at 37°C for 4 days and then diluted to the final concentration with saline just before the experiment. Alzheimer's disease mouse model was prepared by injection (i.c.v.) of "aggregated" A β _{25–35} as previously report [14]. After mice were anesthetized with 2% chloral hydrate (20 mL/kg), they were placed in a stereotaxic device (Kopf Instruments, Tujunga, CA, USA). A 26-gauge single guide cannula (Plastics One, Roanoke, VA, USA) was implanted into the right lateral ventricle (0.3 mm posterior 1.0 mm lateral, and 2.5 mm ventral to bregma). After surgery, a 28-gauge dummy cannula (Plastics One) was inserted into each guide cannula. A β _{25–35} (9 nmol/3 μ L/mouse) was injected with a stepper-motorized micro-syringe (Stoelting, Wood Dale, IL, USA) at a rate of 0.5 μ L/min. Control mice were given an equal volume of vehicle. The injection site was confirmed in preliminary experiments. Neither insertion of the needle nor injection of the saline had a significant influence on survival, and behavioral responses or cognitive functions.

Drug Administration

Simvastatin tablets (Zocor, Merck Sharp & Dohme Limited, Hoddesdon, Herts, UK) were consecutively intragastrically administered for 11 days. SV was once daily administered at a dose of 40 mg/kg/day and was firstly administered at 24 h after A β _{25–35}-injection. α 7nAChR antagonist methyl-lycaconitine (MLA) (0.1 nmol/mouse, i.c.v.), MAPK/ERK kinase (MEK) inhibitor U0126 (0.3 nmol/mouse, i.c.v.) and PI3K inhibitor LY294002 (0.3 nmol/mouse, i.c.v.) were dissolved in DMSO, and then in 0.9% saline with a final concentration of 1% DMSO. These antagonists were injected 30 min before SV-administration. Control mice were given an equal volume of vehicle.

Concerning the difference in physiologic and metabolic processes between human and rodent, the dosage of SV used here is higher than that taken by human subjects if the dosage is

expressed per unit of body weight [18]. This issue also exists in previous studies reporting the neuroprotection or anti-amnesic effect of SV against A β -toxicity and the concentrations of SV used in animal studies are different from the dosage used when treating human patients [7,8]. In this study, 40 mg/kg/day SV was used because this dosage has been used in other studies examining systemic or central effects of SV in mice and chronic administration of SV at this dosage improves memory in adult AD transgenic mice [7,19,20]. Other concentrations of antagonists were chosen as previously reported [15].

Behavior Analysis

Morris water maze task was performed on day 5 to day 9 after A β _{25–35}-injection. In water maze task, a black plastic pool (diameter = 120 cm) was prepared, with water temperature maintained at 20 $\pm$ 1°C. Swimming paths were monitored with a video camera (AXIS-90 Target/2; Neuroscience). Mice were given 90 seconds to reach the hidden platform which was put 1 cm below the water surface. If the mouse did not find the platform within 90 seconds, the trial was terminated and the mouse was put on it for another 30 seconds. Starting position was chosen pseudorandomly and each mouse was trained with three trials per day. All parameters were recorded and analyzed where appropriate. The probe trial was performed on day 10 after A β _{25–35}-injection in which the platform was removed from the maze, and the percent time spent in each quadrant was assessed. Each group contained eight mice.

Y maze task was performed on day 8 after A β _{25–35}-injection in which the maze was made of black painted wood. Each arm was 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually and arm entry was considered to be completed when the hind paws of the mouse were completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The percentage alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two), multiplied by 100. Each group contained eight mice.

Histological Examination

Histological examination of hippocampal CA1 region was assessed on day 11 after A β _{25–35}-injection. After performing Morris water maze task, mice were anesthetized and perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% para-formaldehyde. Brains were removed and immersed in fixative (4°C overnight), and then processed for paraffin embedding. Coronal sections (5 μ m) were cut from the level of hippocampus for toluidine blue and Hoechst staining. In toluidine blue staining, the pyramidal cells in hippocampal CA1 region were identified using a conventional light microscope (DP70, Olympus Optical Co., LTD, Tokyo, Japan) with a 40 $\times$ objective. In Hoechst staining, the slices were stained with Hoechst-33342 and Hoechst-positive cells were counted using a fluorescence-microscope (Olympus PD70) with a 40 $\times$ objective. Density of surviving neurons or Hoechst-positive

cells (die cells) was counted in six sections per mouse and expressed as the number of cells per one mm length along the hippocampal CA1 pyramidal layer [21].

Western Blot Analysis

Western blot analysis was performed on day 5 after A β_{25-35} -injection. After mice were decapitated, the hippocampus was taken quickly and then was homogenized in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM phenyl-methylsulfonyl fluoride and protease inhibitor cocktail (Complete; Roche, Mannheim, Germany). Protein concentration was determined with BCA Protein Assay Kit (Pierce, Rochford, IL, USA). Total proteins (20–40 mg) were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyphosphorylated difluoride (PVDF) membrane. The membranes were incubated with 5% bovine serum albumin or 5% nonfat dried milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 60 min at room temperature, and then were incubated with a mouse monoclonal antiphosphor Akt antibody (1:1000, Cell Signaling), a mouse monoclonal antiphosphor ERK1/2 antibody (1:1000, Cell Signaling) at 4°C overnight. After being washed with TBST for three times, the membranes were incubated with an HRP-labeled secondary antibody, and developed using the ECL detection Kit (Amersham Biosciences, Piscataway, NJ, USA). Following visualization, the blots were stripped by incubation in stripping buffer (Restore, Pierce Chemical Co, Rockford, IL, USA) for 5 min, re-blocked for 60 min with 5% nonfat dried milk at room temperature, then incubated with antitotal ERK1/2 (1:1000, Cell Signaling), Akt (1:1000, Cell Signaling). Western blot bands were scanned and analyzed with the image analysis software package, National Institutes of Health Image. Hippocampal samples were collected from the hemisphere of eight mice as a set of western blot analysis.

Electrophysiological Analysis

Electrophysiological recording was performed on day 9 and day 10 after A β_{25-35} -injection. After performing Y maze task, mice were decapitated and the brains were rapidly removed and the coronal brain slices (400 μ m) were cut using a vibrating microtome (Microslicer DTK 1500, Dousaka EM Co, Kyoto, Japan) in ice-cold oxygenated (95% O₂/5% CO₂) modified artificial cerebrospinal fluid (mACSF) composed of (in mM) NaCl 126, CaCl₂ 1, KCl 2.5, MgCl₂ 1, NaHCO₃ 26, KH₂PO₄ 1.25, and D-glucose 20. After one hour recovery, hippocampal slices were transferred to a recording chamber.

For recording field excitatory postsynaptic potential (fEPSP), slices were perfused continually with the oxygenated mACSF at the temperature of 30 $\pm$ 1°C. A glass microelectrode with the resistance of 4–5 M Ω filled with 2 M NaCl was inserted into the stratum radiatum region of CA1 area. fEPSP was generated by stimulating the Schaffer collateral/commissural pathway using a stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). Stimulus pulses (0.1 ms duration) were delivered every 15 seconds. Signals were obtained using an Axoclamp 2B amplifier (Axon Instru-

ments, Foster City, CA, USA), sampled at 20 kHz and filtered at 10 kHz, and the output was digitized with a Digidata 1200 converter (Axon Instruments). The basal synaptic transmission was recorded using the stimulation intensity yielding the half-maximal fEPSP slope for a given slice. Long-term potentiation (LTP) was induced by high-frequency stimulus (HFS, 100 pulses at 100 Hz) with the same stimulation intensity as pre-HFS. Single pulse recording resumed immediately following HFS and continued for 60 min. A series of fEPSPs slope post-HFS (55–60 min) 20% larger than pre-HFS (15–20 min) baseline and lasting for over 60 min was scored as LTP.

Data Analysis

Data are expressed as means $\pm$ SEM and were analyzed with pClamp (Axon Instruments) and State7 software (StataCorp LP, College Station, TX, USA) software. ANOVAs followed by the Bonferroni's post hoc test was used for statistical analysis with the significant level set at $P < 0.05$.

Results

Effect of SV on A β_{25-35} -Impaired Cognition

The effect of SV on long-term memory in A β_{25-35} -mice was explored using Morris water maze task. As shown in figure 1A, the escape-latency to reach the hidden platform was prolonged in A β_{25-35} -mice when compared to that in control mice ($P < 0.01$ on the 5th day after training). The prolongation of escape-latency in A β_{25-35} -mice was markedly attenuated by administration of SV ($P < 0.01$ on the 5th after training). Here, the swimming speed was unaffected by either A β_{25-35} -injection or SV-administration ($P > 0.05$).

After the hidden-platform was removed from the pool, A β_{25-35} -mice were not able to remember the location of the platform and spent significantly less time in the target quadrant than did the control mice ($P < 0.01$). Notably, after treatment with SV, A β_{25-35} -mice presented better performance in the probe trail test than did saline-treated A β_{25-35} -mice ($P < 0.01$) (Figure 1B).

Spontaneous alternation behavior, which is regarded as a measure of short-term memory, was investigated using Y maze task on day 8 after A β_{25-35} -injection. Figure 1C shows that A β_{25-35} -mice displayed significantly impaired memory, which was rescued by SV-administration. In contrast, the number of arm entries did not change among all the experimental groups, demonstrating that general locomotor activity was not affected by A β_{25-35} -injection or SV-administration ($P > 0.05$). These results demonstrate that SV improves A β_{25-35} -impaired long-term and short-term memory.

Effect of SV on A β_{25-35} -Induced Apoptosis of Pyramidal Cells

We then explored whether SV had protection on hippocampal CA1 pyramidal cells, which is related to the cognitive function, on day 11 after A β_{25-35} -injection. Figure 2A shows that the number of pyramidal cells was reduced approximately

Figure 1 Effect of SV on behavioral performance in A β_{25-35} -mice. **(A)** Morris water task shows that the escape-latency for A β_{25-35} -mice (43.27 ± 6.64 seconds, on the 5th day after training) was longer than that for control mice (15.60 ± 4.10 seconds, on the 5th day after training) ($P < 0.01$). After SV-treatment, the latency on the 5th day after training for A β_{25-35} -mice was 23.91 ± 4.32 seconds ($P < 0.01$ vs. vehicle-treated A β_{25-35} -mice). **(B)** Probe trial shows that A β_{25-35} -mice spent less time in the target quadrant than did control mice. SV treatment markedly increased the percentage time in training-quadrant of A β_{25-35} -mice. $**P < 0.01$ versus control mice, $***P < 0.01$ versus vehicle-treated A β_{25-35} -mice. **(C)** Spontaneous of alternation in Y maze task markedly decreased in A β_{25-35} -mice. Administration of SV increased the percentage of alternation in A β_{25-35} -mice. $**P < 0.01$ versus control mice, $***P < 0.01$ versus vehicle-treated A β_{25-35} -mice. Each group data contained eight mice.

$34.02 \pm 2.01\%$ by A β_{25-35} -injection ($P < 0.01$). After administration of SV, the surviving pyramidal cells in A β_{25-35} -mice markedly increased ($P < 0.05$). The neuronal survival in control mice was not affected by SV-administration. Figure 2B shows that there were more apoptotic cells (Hoechst-positive cells) in A β_{25-35} -mice, which could be reduced by administration of SV. These results demonstrate direct protection of SV against A β_{25-35} -neurotoxicity *in vivo*.

Involvement of $\alpha 7$ nAChR in SV-Increased Akt and ERK Phosphorylation in A β_{25-35} -Mice

There is evidence that A β_{25-35} -induced neurotoxicity in hippocampal CA1 pyramidal cells is mediated through the reduction of Akt and ERK phosphorylation [22,23]. As shown in Figure 3A, SV rescued the reduction of phosphorylation level of Akt (p-Akt) in hippocampus of A β_{25-35} -mice. Besides this, the phosphorylation level of hippocampal ERK2 (p-ERK2) was lower in A β_{25-35} -mice, which was rescued by SV (Figure 3B). It was also found that administration of $\alpha 7$ nAChR antagonist MLA significantly attenuated SV-improved p-Akt and p-ERK in A β_{25-35} -mice (Figure 3A, B). Here, the neuroprotection of SV in A β_{25-35} -mice was markedly blocked by administration of MLA or PI3K inhibitor LY294002. In contrast, administration of MEK inhibitor U0126 did not have such effect (Figure 3C). These results indicate that $\alpha 7$ nAChR-mediated PI3K/Akt signaling pathway is likely involved in SV-induced neuroprotection against A β_{25-35} -toxicity.

Involvement of $\alpha 7$ nAChR-Mediated PI3K/Akt and ERK Signaling Pathways in SV-Rescued LTP in A β_{25-35} -Mice

We then examined the effect of SV on the basal transmission of Schaffer collaterale-CA1 synapse in A β_{25-35} -mice. Input/output (I/O) curve shows that the slope of fEPSP in slices obtained from A β_{25-35} -mice significantly decreased compared to that from control mice ($P < 0.01$), indicating the impaired synaptic transmission. After administration of SV, A β_{25-35} -induced synaptic dysfunction was markedly rescued ($P < 0.01$, Figure 4A).

We also found that HFS evoked a stable potentiation of fEPSP slope in control slices ($143.29 \pm 8.81\%$ at 60 min post-HFS, $n = 14$ slices/eight mice), but failed to induce the potentiation of fEPSP slope in A β_{25-35} -mice slices ($102.13 \pm 7.54\%$ at 60 min post-HFS, $n = 14$ slices/eight mice, Figure 4B, C). After administration of SV, LTP could be induced in A β_{25-35} -mice ($145.17 \pm 6.01\%$ at 60 min post-HFS, $n = 15$ slices/eight mice, Figure 4D). It was also found that after administration of MLA, LY294002, or U0126, the slopes of fEPSP markedly decreased and LTP could not be induced in slices from SV-treated A β_{25-35} -mice (Figure 5). Collectively, our results indicate an involvement of $\alpha 7$ nAChR-mediated PI3K/Akt and ERK signaling pathways in SV-rescued LTP in A β_{25-35} -mice.

Involvement of $\alpha 7$ nAChR-Mediated PI3K/Akt and ERK Signaling Pathways in Antiamnesic Effect of SV in A β_{25-35} -Mice

As shown in figure 6, the antiamnesic effects of SV on the prolongation of escape-latency and decrease of swimming time in the target quadrant in Morris water maze task were markedly attenuated by application of MLA or LY294002. In SV-treated A β_{25-35} -mice, the increase in alternation behavior in Y maze task was also attenuated by MLA or LY294002. Here, it was noted that application of U0126 also blocked the antiamnesic effect of SV but exerted less effect on SV-action than did MLA or LY294002.

Figure 2 Neuroprotective effect of SV on A β_{25-35} -induced neuronal death. **(A)** The death of pyramidal cells in hippocampal CA1 region was examined using Toluidine blue staining. Healthy CA1 pyramidal neurons showed a round cell body with a plainly stained nucleus. Bar graph represents the number of surviving pyramidal cells. ** $P < 0.01$ versus control mice, # $P < 0.05$ versus vehicle-treated A β_{25-35} -mice. **(B)** Representative images of Hoechst staining in hippocampal CA1 region of control (left), A β_{25-35} -mice (middle) and SV-treated A β_{25-35} -mice (right). Each group data contained eight mice.

Discussion

A chronic cerebral hypoperfusion is supposed to play an important role in neurodegeneration and amnesia in AD. The decrease in cerebral blood flow has been correlated with AD progression and with response to therapy [24,25]. These reports support the notion that statins, a kind of broadly used cholesterol-lowering drugs, have therapeutic potential in AD [26]. The present results showed that administration of SV for 11 days could improve the cognitive deficits, decrease the neuronal apoptosis and rescue LTP induction in A β_{25-35} -mice (Figure 1, 2, 4). The neuroprotection of SV against A β -toxicity was sensitive to $\alpha 7$ nAChR-cascaded PI3K/Akt signaling pathway (Figure 2). The rescue of LTP in SV-treated A β_{25-35} -mice depended on $\alpha 7$ nAChR-PI3K/Akt and ERK signaling pathways (Figure 5).

Chronic SV-treatment improves the behavior performance in transgenic AD model mice independent of decreasing brain A β level or plaque load [7,8]. Moreover, an enhancement of spatial learning and memory in normal mice by SV-treatment is consistent with this concept [8]. A variety of mechanisms have been proposed to explain how statins display favorable effects in AD, such as increased expression of Akt, endothelial nitric oxide synthase and c-Fos or Egr-1 [7,8]. This study found that administration of SV, a lipophilic statin that is able to transverse the blood brain barrier, markedly attenuated the hippocampal neuronal death induced by A β_{25-35} -injection (Figure 2), first providing *in vivo* evidence of direct neuroprotection of SV against A β -toxicity.

The down-regulation of $\alpha 7$ nAChRs in hippocampus and cortex is the most initial disruption of cholinergic system in AD, which correlates well with A β -induced neurotoxicity [27]. Activation of

Figure 3 Involvement of $\alpha 7$ nAChR-PI3K/Akt signaling pathway in SV-induced neuroprotection in A β_{25-35} -mice. **(A and B)** p-Akt (A) and p-ERK (B) decreased in A β_{25-35} -mice, which could be rescued by SV-treatment. Administration of $\alpha 7$ nAChR antagonist MLA markedly blocked SV-action in A β_{25-35} -mice. The densitometric values for p-Akt and p-ERK2 were first normalized by the protein amounts of Akt and ERK2, and then normalized by the basal values of p-Akt/Akt and p-ERK2/ERK2 in control mice, respectively. ** $P < 0.01$ versus control mice; ## $P < 0.01$ versus vehicle-treated A β_{25-35} -mice, ** $P < 0.01$ versus SV-treated A β_{25-35} -mice, * $P < 0.05$ versus SV-treated A β_{25-35} -mice. **(C)** SV-neuroprotection on hippocampal CA1 pyramidal cells in A β_{25-35} -mice was attenuated by antagonism of $\alpha 7$ nAChR and PI3K. In contrast, the neuroprotection of SV was unaffected by MEK inhibitor. ** $P < 0.01$ versus control mice; ## $P < 0.01$ versus vehicle-treated A β_{25-35} -mice; ** $P < 0.01$ versus SV-treated A β_{25-35} -mice.

$\alpha 7$ nAChRs has been proved to ameliorate A β -induced hippocampal neuronal death [9,15]. It is reported that A β_{1-40} prevents the stimulation of sympathetic $\alpha 7$ nAChR to cause the release of nitric oxide in parasympathetic nitrergic nerves and subsequent vasodilatation, which can be reversed by mevastatin and lovastatin [28]. Here, administration of $\alpha 7$ nAChR antagonist significantly decreased the surviving cell in hippocampal CA1 area in SV-treated A β_{25-35} -mice (Figure 3). Combined with these reports, it is indicated that $\alpha 7$ nAChR may be involved in the protection of SV against A β -neurotoxicity. Cholesterol is a very abundant component of the membrane where AChR is located [29]. SV reduces

cholesterol level by inhibiting HMG-CoA reductase activity. However, there is evidence that the effects of statins on $\alpha 7$ nAChR are likely independent of lipid-lowering action. For example, Guan's research group report that statins treatment can upregulate $\alpha 7$ nAChR mRNA and protein in cultured neurons and astrocytes, but pretreatment of lovastatin fails to inhibit the effect of cholesterol on $\alpha 7$ nAChR expression [11,30]. Besides this, statins prevent cholinesterase inhibitors (ChEIs)-induced inhibition in $\alpha 7$ nAChR and this effect is seen on concurrent administration of statins (lovastatin and mevastatin) with ChEIs, which suggests that statins protect $\alpha 7$ nAChR function directly at the receptor level [12]. As

Figure 4 Effect of SV on long-term potentiation (LTP) induction in A β_{25-35} -mice. **(A)** Slopes of EPSP are plotted against stimulus intensity ranging from 0.1 to 1.1 mA. The correlation coefficient (b) of the regression line for I/O curve in A β_{25-35} -mice ($b = 0.88$) was markedly smaller than that in control group ($b = 1.31$) ($P < 0.01$). After SV-treatment, the correlation coefficient in A β_{25-35} -mice significantly increased ($b = 1.27$). Data for each point consisted of 14 slices derived from eight mice. **(B and C)** HFS (denoted by $\uparrow$) induced LTP in control mice (B) but failed to induce LTP in A β_{25-35} -mice (C). **(D)** After treatment with SV, HFS induced LTP in slices obtained from A β_{25-35} -mice.

Figure 5 Involvement of $\alpha 7$ nAChR and PI3K signaling pathway in SV-rescued LTP induction in A β_{25-35} -mice. **(A)** SV-enhanced fEPSP in A β_{25-35} -mice was markedly blocked by MLA, U0126, or LY294002. $*P < 0.05$ versus SV-treated A β_{25-35} -mice, $**P < 0.01$ versus SV-treated A β_{25-35} -mice; stimulation intensity was 0.5 mA. **(B)** LTP could not be induced in A β_{25-35} -mice by coadministration of SV and MLA. **(C)** SV-rescued LTP induction in A β_{25-35} -mice was markedly inhibited by administration of U0126. **(D)** Administration of LY294002 inhibited LTP induction in SV-treated A β_{25-35} -mice.

discussed above, SV may upregulate $\alpha 7$ nAChR expression or modulate $\alpha 7$ nAChR function directly which are impaired upon A β -toxicity. More experiments are needed to confirm whether (and how) SV modulates $\alpha 7$ nAChR.

A β -neurotoxicity in hippocampal CA1 pyramidal neurons is mediated through down-regulating PI3K/Akt and ERK signaling pathways which play an important role in cell growth, survival

and proliferation [22,23]. $\alpha 7$ nAChR, in particular, contributes to activation of PI3K/Akt pathway, which is important for the neuroprotection of $\alpha 7$ nAChR against A β -toxicity [15,23]. This study found that A β_{25-35} -induced decrease of p-Akt and p-ERK was markedly rescued by SV, which was sensitive to $\alpha 7$ nAChR antagonist. Moreover, the antagonists of $\alpha 7$ nAChR and PI3K, but not of MEK markedly attenuated SV-improved surviving cell in A β_{25-35} -

Figure 6 Effect of α 7nAChR, PI3K, and MEK inhibitors on antiamnesic effect of SV in A β ₂₅₋₃₅-mice. **(A)** The histogram shows the latency on the 5th day after training in Morris water maze task. Following administration of MLA, LY294002 or U0126, the latency in SV-treated A β ₂₅₋₃₅-mice was significantly prolonged. ** P < 0.01 versus control mice; ## P < 0.01 versus vehicle-treated A β ₂₅₋₃₅-mice, ** P < 0.01 versus SV-treated A β ₂₅₋₃₅-mice. **(B)** The percentage time in training quadrant in SV-treated A β ₂₅₋₃₅-mice was decreased by administration of MLA, LY294002 or U0126. ** P < 0.01 versus control mice; ## P < 0.01 versus vehicle-treated A β ₂₅₋₃₅-mice, ** P < 0.01 versus SV-treated A β ₂₅₋₃₅-mice, * P < 0.05 versus SV-treated A β ₂₅₋₃₅-mice. **(C)** SV-improved percentage of alternation in A β ₂₅₋₃₅-mice in Y maze task was also markedly attenuated by MLA or LY294002 or U0126. ** P < 0.01 versus control mice; ## P < 0.01 versus vehicle-treated A β ₂₅₋₃₅-mice, ** P < 0.01 versus SV-treated A β ₂₅₋₃₅-mice, * P < 0.05 versus SV-treated A β ₂₅₋₃₅-mice.

mice (Figure 3). These results indicate that SV may target α 7nAChR to activate PI3K/Akt and ERK, but only α 7nAChR-PI3K/Akt signaling pathway is involved in the neuroprotection of SV against A β -toxicity. On the other hand, although statins are reported to activate PI3K-Akt and ERK pathways [31,32], *in vitro* evidence shows that the neuroprotection of pretreatment with SV against A β -induced toxicity is not able to activate Akt or ERK2 [33]. This discrepancy may be due to the difference in AD model types and dosage or time of SV-treatment. Of course, besides α 7nAChR-PI3K/Akt pathway, the neuroprotection of SV may be mediated through other mechanisms such as decreasing A β -induced intracellular calcium rise, accumulation of reactive oxygen species and caspase-3 activity [33].

Synapse damage occurs during the early stage of AD, which is correlative with cognitive decline [34]. This study showed that LTP could be induced in SV-treated A β ₂₅₋₃₅-mice (Figure 4), implying that SV rescues A β -impaired hippocampal synaptic plasticity. Activation of ERK signaling pathway in hippocampus is important for LTP induction [35]. Here, HFS failed to induce LTP in A β ₂₅₋₃₅-mice if they were coadministered with SV and U0126 (Figure 5), which indicates that ERK signaling pathway is responsible for SV-rescued synaptic plasticity that was impaired by A β ₂₅₋₃₅-injection. Several research groups have shown that A β -induced blockade of α 7nAChR can depress synaptic transmission and impair LTP induction [9,10]. There is evidence that PI3K/Akt pathway is important for hippocampal LTP induction and the level of p-Akt in the hippocampus is in parallel with spatial memory formation [36]. Li's research group reports that SV-enhanced hippocampal LTP in C57BL/six mice depends on the activation of Akt [37]. Here, administration of α 7nAChR or PI3K antagonist significantly blocked LTP in SV-treated A β ₂₅₋₃₅-mice (Figure 5), suggesting that α 7nAChR and PI3K/Akt pathway are probably involved in SV-rescued synaptic plasticity. As discussed above, SV-increased p-Akt may be mediated through α 7nAChR. Besides this, SV enhances hippocampal LTP through inhibiting farnesylation to augment the recruitment of PI3K activity [38]. Although pre-treatment with SV prevents A β -induced synapse damage *in vitro* [39], *in vivo* study reports that chronic SV-treatment did not affect synaptic markers PSD-95, synaptophysin, or the NMDA receptor subunit NR2B in synaptosomal P2 fractions from cortex and hippocampus in adult and aged AD mice [7]. More experiments are needed for exploring the mechanisms underlying SV-rescued synaptic plasticity in AD.

In summary, this study showed that blockage of α 7nAChR, PI3K, and MEK markedly attenuated SV-improved behavior performance in A β ₂₅₋₃₅-mice (Figure 6), indicating that SV improves A β -induced deficit in learning and memory through direct neuroprotection and rescue of synaptic plasticity. α 7nAChR is a potential target of SV and SV may exert its antiamnesic effect through modulating PI3K/Akt and MAPK/ERK signaling pathways. This study provides a possible cellular basis for the beneficial effect of SV on A β -impaired cognition function.

Acknowledgments

We thank Dr. Edith Hamel and Dr. Xin-Kang Tong for the helpful discussion on the manuscript. This work was supported by Fonds de recherche Santé-National Natural Science Foundation of China

Collaboration (812111370), National Natural Science Foundation of China (31271206) and Science and Technology Project of Jiangsu Province (BK2011029).

Conflict of Interest

The authors declare no conflict of interest.

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