Davunetide improves spatial learning and memory in Alzheimer's disease-associated rats

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HIGHLIGHTS

- Davunetide protected against A β -42 induced impairment of spatial learning and memory in rats
- Davunetide reversed A β -induced suppression of hippocampal LTP in rats
- Davunetide mostly restored the level of phosphorylated AKT in rat hippocampus

ARTICLE INFO

Article history:

Received 28 December 2016

Received in revised form 25 February 2017

Accepted 27 February 2017

Available online 28 February 2017

Keywords:

Davunetide

Amyloid β protein

Spatial memory

Long term potentiation

Phosphorylated AKT

Synaptic plasticity

ABSTRACT

Memory loss and cognition decline are the main clinical manifestations of Alzheimer's disease (AD). Amyloid β protein (A β) aggregated in the brain is one of the key pathological characteristics of AD and responsible for the deficits in learning and memory. It is reported that davunetide, an octapeptide derived from activity-dependent neuroprotective protein (ADNP), inhibited A β aggregation and A β -induced neurotoxicity. To further characterize the neuroprotective roles of davunetide and its possible mechanism, the present study investigated the effects of davunetide on A β 1-42-induced impairments in spatial memory, synaptic plasticity and hippocampal AKT level. In Morris water maze (MWM) test, bilateral intrahippocampal injection of A β 1-42 significantly increased escape latency and decreased target quadrant swimming time of rats, while three weeks of intranasal application of davunetide reversed the A β 1-42-induced learning deficits and memory loss in a dose-dependent manner. In vivo field potentiation recording showed that A β 1-42 suppressed long-term potentiation (LTP) of excitatory postsynaptic potential (fEPSP) in the hippocampal CA1 region of rats, while davunetide effectively blocked the suppression of LTP, without affecting paired-pulse facilitation (PPF). Western blotting experiments showed a significant decrease in the level of hippocampal p-AKT (Ser473), not total AKT, in A β 1-42 only group, which was mostly antagonized by davunetide treatment. These findings demonstrate that davunetide, probably by enhancing PI3K/AKT pathway, plays an important positive role in attenuating A β 1-42-induced impairments in spatial memory and synaptic plasticity, suggesting that davunetide could be an effective therapeutic candidate for the prevention and treatment of neurodegenerative disease such as AD.

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1. Introduction

The main manifestation of Alzheimer's disease (AD) in the early stage is gradual deficits in the cognitive function. With the development of the disease, spatial disorientation, loss of motivation, abnormal behaviors, and serious dementia gradually appeared [1]. One of the pathological hallmarks of AD is high density of senile plaques (SPs), which

aggregated in the brain especially in the hippocampus and cerebral cortex. The main component of SP is amyloid β protein (A β) derived from amyloid precursor protein (APP) [2]. In two forms of natural A β , i.e. A β 1-40 and A β 1-42 [3], A β 1-42 is easier to aggregate early, and has more neurotoxicity than A β 1-40 [4]. Moreover, A β oligomers rather than A β fibrils have more neurocytotoxicity [5,6]. Many studies have confirmed that intracerebral injection of A β 1-42 could damage spatial memory and synaptic transmission of animals [7,8]. Our previous studies also found that A β 1-42 injection inhibited hippocampal long term potentiation (LTP) in rat [9,10]. However, the molecular mechanisms underlying A β toxicity remain to be clarified and the effective neuroprotectants against A β are urgently needed to be explored.

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Activity-dependent neuroprotective protein (ADNP) was originally isolated from conditioned medium of astrocytes treated with vasoactive intestinal peptide (VIP). Thereafter, ADNP has been shown to have potent neuroprotective actions among the neurotrophic factors secreted by glial cells [11]. In human and mouse brains, ADNP is predominantly expressed in the cerebellum, hippocampus and cerebral cortex [12]. Moreover, the expression of ADNP in the brain has been related to not only the neurogenesis in the hippocampus but also the early or mild AD progression. For example, ADNP expression was higher in 6-month-old PS1xAPP mice hippocampus than in WT mice [13]. A most recent study showed a significant association between greater cortical A β and lower ADNP mRNAs. This supported that ADNP is essential for brain formation and linked to cognitive functions [14]. Davunetide (also namely NAP), an eight amino acid (NAPVSIPQ) neuroprotective peptide, is derived from ADNP, which has similar structural and functional characteristics to the active core of ADNP. It has been confirmed that davunetide has potent biological activities. Low concentration davunetide protected astrocytes against zinc toxicity [15], promoted neurite outgrowth in PC-12 cells and hippocampal neurons and increased synaptophysin expression in both rat hippocampal and cortical cultures [16,17]. A most recent article reported that ADNP/NAP dramatically increased microtubule end-binding protein-Tau interaction, suggesting a novel target for endogenous ADNP neuroprotection [18]. Also, davunetide prevented aggregation of A β peptide *in vitro* [19] and A β -induced cellular toxicity in rat cerebral cortex neurons [16,17]. It seems that davunetide has beneficial effects on the neurodegenerative disorders such as AD.

However, the *in vivo* neuroprotective effects of davunetide against neurotoxic A β and its molecular mechanism remain largely unknown. The present study, therefore, first investigated the protective effects of chronic administration of davunetide on A β 1–42-induced impairments in spatial learning and memory of rats by using Morris water maze (MWM) tests. Then, we explored the probable electrophysiological and molecular mechanisms by recording *in vivo* hippocampal long-term potentiation (LTP) and examining PI3K/AKT signal pathway in the hippocampus with field potential and western blotting techniques.

2. Materials and methods

2.1. Animals and drugs

All experiments were conducted on male Sprague-Dawley (SD) rats aged between 3 and 4 months. The rats, provided by the Beijing Experimental Animal Center of Military Medical Sciences, were housed in cages with *ad libitum* access to food and water, 12 h light/dark cycle and 20–25 °C temperature. All animal procedures were conducted in accordance with the Guidelines for Care and Use of Laboratory Animals and were approved by the Institutional Animal Research Ethics Committee at Shannxi Medical University. Amyloid β peptide (A β 1–42) was ordered from Abcam (UK). Davunetide was synthesized by the Shanghai GL Biochem Ltd. (Shanghai, China) with a purity of 99.37% detected by reversed-phase high performance liquid chromatography (RP-HPLC). A β 1–42 oligomer solution was prepared by the following procedures: dissolving A β 1–42 in 100% HFIP at a concentration of 1 mg/ml; incubating the solution at 20–25 °C for 1 h under vortexing at a medium speed; sonicating the solution in a water bath sonicator for 10 min; after dividing into small volume and drying with nitrogen gas, quantitative A β 1–42 was stored at –80 °C [9,20]. DMSO was used to resuspend the A β 1–42 before using. After D-PBS was added for a working solution 2.5 μ g/ μ l, the solution was incubated with occasional vortexing at a medium speed and left at 4 °C for 24 h to allow for peptide aggregation into oligomers [21].

2.2. Surgical and drug administration

After one week of acclimation, SD rats were randomly divided into four groups ($n = 10$ for each group): vehicle + saline, vehicle + A β 1–42, Davunetide + saline, and Davunetide + A β 1–42. The protocol of bilateral hippocampal injection was previously described [22,23]. The animal was anesthetized using 5% chloral hydrate and fixed on a digital display stereotaxic instrument (RWD Life Science, China). 2 μ l A β 1–42 solution (or vehicle) was injected into the bilateral hippocampus of rats by micro-injection pump (KD Scientific, USA) connecting a quartz capillary injection tube. The coordinates of injection tube tip were: anterior-posterior, –3.0 mm; medial-lateral, –2.2 mm; dorsoventral, –3.0 mm to the bregma as a reference point. Intranasal administration of davunetide: from the next day after surgical, animals received a daily intranasal injection of davunetide (2.5 μ g in 10 μ l, half in each nostril) or saline (10 μ l) [24,25] for 3 weeks. During behavioral tests, daily davunetide administration was still carried on.

2.3. Morris water maze test

Classical Morris water maze (MWM) test was used to detect animal's ability of long term spatial memory. The water maze apparatus is a circular stainless steel basin (150 cm in diameter and 60 cm deep) with different cues on the inner surface painted black. The maze was filled with water at a temperature of 22 °C [26]. A CCD camera was installed right above the sink to record the animal's swimming track. Behavior software (Ethovision 3.0, Noldus Information Technology, Netherlands) was used to analyze swimming distance and time of animals. Hidden platform acquisition was performed firstly in consecutive five days to detect the learning ability of rats [27]. An escape platform (diameter 10 cm) was placed in the first quadrant (the target quadrant) of four quadrants divided by software, and was submerged 1–2 cm below the surface. Rats were put into the maze at the midpoint of the maze margin facing the wall. Each rat was trained four times a day. Memory tests were conducted by probe trial after withdrawing escape platform on the sixth day. Each animal was tested twice and limited for 120 s in each trial. The vision and motor function of animals were checked by a visual platform test, in which the escape platform was elevated 2 cm above the water and each animal was tested twice [28].

2.4. *In vivo* hippocampal LTP recording

Hippocampal LTP is an important indicator of neuronal plasticity. The LTP recording was performed *in vivo* with the same animal after undergoing behavioral tests. The rats were anesthetized with urethane (1.3 g/kg, *i.p.*), and a small hole (2.5 mm diameter) was drilled on the skull for inserting a pair of bounded stimulation/recording electrodes. The body temperature of rats was maintained at 37 ± 0.5 °C with a heating pad throughout experimental period. The tip of concentric bipolar stimulating electrode (FHC, Inc., USA) was driven downward by a micromanipulator to the Schaffer collateral/commissural pathway with the following coordinates (using the bregma as a reference point): AP, –4.2 mm; ML, –3.8 mm [29]. The tip position of recording electrode is the stratum radiatum in the hippocampus CA1 region with the following coordinates: AP, –3.4 mm; ML, –2.5 mm. The final vertical positions of the electrodes in the hippocampus were determined by electrophysiological criterion, an obvious negative deflecting field excitatory postsynaptic potential (fEPSP) evoked by a stimulus from stimulation isolator (Master-9, AMPI, Israel). The basal fEPSPs were evoked by continuous single electric stimulus (intensity: elicited 50% of maximal response; frequency: 0.033 Hz) and recorded for 30 min. LTP of fEPSPs was induced by high-frequency (200 Hz) stimulation (HFS) containing 20 pulses. A > 30% increase in the amplitude of fEPSP after HFS was considered successful induction of LTP. After that, fEPSPs were recorded at a frequency of 0.033 Hz for at least 60 min. To determine whether presynaptic mechanisms were involved, paired pulse

facilitation (PPF) was also examined with paired pulses after recording basal fEPSPs. All signals from recording electrode were sent to a signal acquisition and processing system (Chengdu Instruments Inc., China) for off-line analysis.

2.5. Western blotting

After electrophysiological experiment, rats were sacrificed under anesthesia. Bilateral hippocampi were removed and homogenized by ultrasonic crusher under cold condition. Homogenates were centrifuged at 14000 rpm for 10 min at 4 °C. The concentration of total protein in the supernatant was determined by Micro bicinchoninic acid (BCA) assay kit (Boster Co. Ltd. China). Protein samples (40 µg) were separated by electrophoresis on 12% SDS-polyacrylamide gels. After electrophoresis, proteins were transferred to PVDF membrane. Membranes were incubated with 5% bovine serum albumin (BSA) at 4 °C conditions for 2 h in order to block nonspecific binding. Then, membranes were incubated with first antibody include anti-p-AKT antibody (Ser473, 1:1000), and anti-AKT antibody (1:1000) at 4 °C for 12 h. Thereafter, membranes were rinsed three times every five minutes by Tris-Buffered Saline with 0.05% Tween 20 (TBST), then incubated with secondary antibody (1:50,000, ZSGB-BIO, Inc. China) at room temperature for 2 h. Afterwards, membranes were again rinsed three times with the same conditions as previously in TBST. The immunocomplexes were visualized by enhanced chemiluminescence using the ECL kit (Beyotime, Inc. China) according to manufacturer instructions. The signals of the membranes were scanned with ChemiDoc XRS + system and quantified with image lab 5.2 software (Bio-Rad, Inc. US). β -actin was used as an internal control for sample loading. AKT and p-AKT were normalized to their corresponding β -actin value.

2.6. Data analysis

All data were expressed as means $\pm$ standard errors (SEM) and analyzed by statistical software SPSS13.0. Data of MWM in training days were calculated and compared with three-way ANOVA. Other data of behavioral experiments were calculated with two-way ANOVA. The data from electrophysiological and western blotting experiments were calculated by using two-way classification ANOVA. Statistical significance was assigned as $P < 0.05$.

3. Results

3.1. Intranasal administration of davunetide protected against A β 1–42 induced impairment of spatial learning and memory in rats

The spatial learning ability of rats was assessed by performing a consecutive 5-day underwater platform test in the MWM. As shown in the Fig. 1A, the mean escape latency of rats to find underwater platform gradually decreased in each group during 5 training days ($F_{(4, 160)} = 200.433$, $P < 0.001$). Significant main effects existed in group saline vs davunetide treatment [$F_{(1, 40)} = 15.145$, $P < 0.01$] and in group vehicle vs A β 1–42 injection [$F_{(1, 40)} = 12.115$, $P < 0.01$] on escape latency. There was a significant interaction between A β 1–42 injection and davunetide treatment [$F_{(1, 40)} = 11.556$, $P < 0.01$]. Tukey's post hoc test showed that the escape latencies of rats in vehicle + A β 1–42 group were significantly increased on the training day 3 (46.43 ± 3.76 s, $P < 0.01$), day 4 (44.34 ± 3.54 s, $P < 0.01$) and day 5 (39.94 ± 3.87 s, $P < 0.001$) compared to that in vehicle + saline group on the training day 3 (31.44 ± 2.84 s), day 4 (24.02 ± 3.40 s), and day 5 (22.54 ± 1.85 s), indicating that A β 1–42 significantly damaged the spatial learning ability of rats. Importantly, davunetide treatment significantly decreased the escape latency of rats, being (35.46 ± 3.06 s, $P < 0.01$), (27.72 ± 4.21 s, $P < 0.01$), (25.27 ± 3.86 s, $P < 0.01$), on day 3–5, respectively, significantly lower than that in vehicle + A β 1–42 group. Typical

swimming traces of all the four group animals on 4th training day were shown in Fig. 1E.

The spatial memory of rats was assessed by two times of prob. trial. In the test, the platform was removed, and each animal was permitted to swim for 120 s. We found that there were significant main effects of davunetide treatment and A β 1–42 injection [davunetide treatment [$F_{(1, 40)} = 2.663$, $P < 0.05$]; A β 1–42 injection [$F_{(1, 40)} = 6.326$, $P < 0.01$] and interaction effects between A β 1–42 injection and davunetide treatment [$F_{(1, 40)} = 7.896$, $P < 0.01$]. As shown in the Fig. 1B, the following Tukey's post hoc test showed that the significant decrease in the percentage of swimming time of rats in vehicle + A β 1–42 group ($17.9 \pm 2.2\%$, $P < 0.01$) compared with the vehicle + saline group ($40.1 \pm 1.6\%$) had an obvious recovery in Davunetide + A β 1–42 group ($36.9 \pm 2.9\%$, $P < 0.05$), compared with that in the vehicle + A β 1–42 group. Fig. 1F showed the typical swimming traces of rats in the probe trials in different groups.

To exclude the impact of motor ability and vision on the above tests, a visible platform test was performed after prob tests. In the visible platform test, the average swimming speed and swimming latency of rats to find the visible platform were compared. The results (Fig. 1) showed that there is no significant difference ($P > 0.05$) in swimming speed (Fig. 1C) and swimming time to visible platform (Fig. 1C), indicating that both A β 1–42 and davunetide did not affect the athletic ability and visual of animals.

3.2. 3.2. Davunetide reversed A β 1–42-induced suppression of hippocampal LTP

After water maze test, we recorded in vivo hippocampal LTP in the CA1 region of the same animals to analyze the association between spatial memory and synaptic plasticity. After 30 min of stable baseline fEPSP recording, three strings of HFS (200 Hz) were delivered to Schaffer collateral, and fEPSPs potentiated in the CA1 region were continuously recorded for 60 min. Fig. 2A showed the changes of fEPSPs slope before and after HFS in different groups. Two-way ANOVA indicated that both davunetide treatment group and A β 1–42 injection group had significant main effects on the induction of LTP at 1 min after HFS [davunetide treatments: $F_{(1, 40)} = 5.483$, $P < 0.05$; A β 1–42 injection $F_{(1, 40)} = 22.164$, $P < 0.001$]. There was also a significant interaction effect between davunetide treatment group and A β 1–42 injection group [$F_{(1, 40)} = 7.833$, $P < 0.01$]. As shown in the Fig. 2B, Tukey's post-hoc test showed that A β 1–42 injection significantly suppressed the induction of LTP ($n = 10$, $P < 0.001$), while davunetide treatments reversed the suppression ($n = 10$, $P < 0.01$). At 60 min after HFS (Fig. 2C), both davunetide treatment group and A β 1–42 injection group had significant main effects on the maintenance of LTP [davunetide treatments: $F_{(1, 40)} = 24.103$, $P < 0.001$; A β 1–42 injection $F_{(1, 40)} = 34.260$, $P < 0.001$]. There was also a significant interaction effect between davunetide treatment group and A β 1–42 injection group [$F_{(1, 40)} = 19.957$, $P < 0.001$] (Fig. 2B). Tukey's post hoc test showed that A β 1–42 injection can significantly suppressed the maintenance of LTP ($n = 10$, $P < 0.001$), which was reversed by davunetide treatments ($n = 10$, $P < 0.001$).

Further, we recorded paired-pulse facilitation (PPF) to clarify whether presynaptic mechanism was involved in the effects of davunetide and A β 1–42 on synaptic plasticity. The ratio of second fEPSP over first fEPSP was compared (Fig. 2D). Statistical results showed that no significant difference between the groups ($n = 10$, $P > 0.05$), suggesting A β 1–42 injection and davunetide treatment did not affect presynaptic neurotransmitter release in the rat hippocampal CA1 region.

3.3. Davunetide mostly restored the level of phosphorylated AKT in rat hippocampus

The PI3K/Akt pathway has attracted attention for its pivotal role in regulating cell survival, proliferation, synaptic plasticity and cognitive

Fig. 1. Davunetide treatments reversed Aβ1-42-induced impairments in spatial learning and memory of rats. (A) Plots showing the average escape latencies of rats in searching for the hidden platform over five consecutive training days. The escape latency was significantly increased in Aβ1-42-treated rats on the 3–5 training days ($n = 10$, $**P < 0.01$, $***P < 0.001$), which was reversed by davunetide treatments ($n = 10$, $**P < 0.01$, Error bars indicate SEM). (B) Histograms showing the average swimming time of rats in the target quadrant, with a significant decrease in Aβ1-42 injection rats compared to the control group ($n = 10$, $**P < 0.01$), which was significantly antagonized by davunetide treatments ($n = 10$, $*P < 0.05$). (C and D) Histograms showing the average swimming speed ($n = 10$, $P > 0.05$) and the average swimming time to visible target ($n = 10$, $P > 0.05$) in all groups. (E) Representative swimming traces of animals on the 4th training day in hidden platform tests. (F) Representative swimming traces of rats in four groups during the probe trials. The large circle represents the Morris water maze pool and the small circle represents the platform.

performance [30,31]. To clarify whether the neuroprotection of davunetide in spatial memory and synaptic plasticity was associated with the regulation of PI3K/Akt signal pathway, we further examined the levels of total AKT and phosphorylated (Ser473) AKT (p-AKT) in the hippocampus. As shown in the Fig. 3 by Western blotting, Two-way ANOVA indicated that there are no main effects on the level of AKT (AKT/β-actin) between Aβ1-42 injection and davunetide treatment [$F_{(1, 24)} = 3.923$, $P > 0.05$], suggesting no significant change in the total levels of Akt protein. However, Aβ1-42 injection and davunetide treatment had significant main effects on the level of p-AKT/AKT [Aβ1-42 injection: $F_{(1, 24)} = 5.267$, $P < 0.05$; davunetide treatment: $F_{(1, 24)} = 9.687$, $P < 0.01$]. Tukey's post hoc test showed that expression level of p-AKT (p-AKT/AKT) in the hippocampus was decreased in Aβ1-42-induced rats ($n = 6$, $P < 0.01$), while davunetide

treatment mostly and significantly rescued the level of p-AKT ($n = 6$, $P < 0.01$).

4. Discussion

Although the mechanism of AD is not fully understood, one of the popular proposals is the amyloid cascade hypothesis. Aβ has been considered the initiator of subsequent events leading to the occurrence of AD [32]. It is well known that the spatial learning and memory function is closely dependent on the hippocampus in mammals, and any insults including neurotoxic Aβ could impair the learning and memory behavior [8,33]. There are two major Aβ isoforms, Aβ1-40 and Aβ1-42, in the brain of AD patients [34]. Although >90% of Aβ isoforms is Aβ1-40, Aβ1-42 oligomers are the most neurotoxic form [35]. In pathological

Fig. 2. Davunetide treatments reversed Aβ1-42-induced hippocampal LTP suppression. (A) Time course of fEPSP change before and after HFS. Insets, typical fEPSP traces recorded from the four groups of rats at time points. (B) Histograms showing that Aβ1-42 significantly suppressed the induction of LTP immediately after HFS (n = 10). Davunetide alone had no effect on the LTP induction (n = 10, $P > 0.05$), but restored the induction of LTP. The recordings at the first 1 min after HFS were used to calculate the fEPSP slope. ** $P < 0.01$, *** $P < 0.001$. (C) Histograms showing that davunetide treatment reversed Aβ1-42-induced suppression in the LTP maintenance (n = 10). The recordings at the last 1 min after HFS were used to calculate the fEPSP slope. *** $P < 0.001$. (D) Histograms showing that Aβ1-42 and davunetide treatments did not affect the PPF (fEPSP2/fEPSP1) in the hippocampal CA1 region. Insets, representative fEPSP trace induced by paired pulses.

conditions, Aβ molecules spontaneously gathered and deposited into soluble oligomers, fibrils and SPs, then caused oxidative damage and disturbance of kinase/phosphatase activity, eventually leading to neuronal death [36]. Therefore, aged Aβ1-42 was used in the present study to mimic the neurodegenerative pathological processes of AD by directly injecting into the bilateral hippocampi of rats. As shown above, our results demonstrated that intrahippocampal injection of Aβ1-42 not

only impaired the spatial learning and memory of rats in MWM tests, but also suppressed LTP of fEPSPs in the hippocampal CA1 region on the same animals. These results are similar to our recent reports [9,10].

In the central and peripheral nervous system, neurotrophic factors (NTFs) is capable of regulating neuronal survival and differentiation, and promoting the growth of new neurons and synapses [37,38]. These NTFs and their receptors, such as brain-derived neurotrophic

Fig. 3. Davunetide treatment inhibited Aβ1-42 induced activation of AKT in rat hippocampus. (A) Representative western blot bands of p-AKT (Ser473), AKT, β-actin in the hippocampus of rats. (B) Aβ1-42 and davunetide did not affect the level of total AKT (n = 6, $P > 0.05$). (C) Aβ1-42 injection significantly decreased the level of p-AKT (Ser473) in saline-Aβ1-42 treated rats, and this deficit was mostly restored by the davunetide treatment (n = 6, ** $P < 0.05$). Error bars indicate SEM.

factor (BDNF) and TrkB receptors, are also active in the hippocampus, cortex, and basal forebrain areas vital to learning, memory and thinking [39]. Many studies have shown lowered levels of NTFs in the AD brain, suggesting that NTFs have a protective role against A β toxicity and a very broad prospect in treating degenerative central nervous system diseases including AD [40,41]. However, NTFs have relatively larger molecular weight, in general, and are difficult to pass through the blood-brain barrier (BBB). For example, nerve growth factor (NGF) is a 130 kDa complex of three proteins and zinc ions, and its active region is beta-NGF, a single chain of two 118 amino acids joined by a non-covalent bond. BDNF consists of 119 amino acid residues and the molecular weight is 13.5 kDa. The molecular weight of ADNP is 124 kDa [42], which greatly limits its clinical application [43]. In recent years, many short peptides were found to have strong effects of nutrition and protection. For instance, Humanin contains 14 amino acid residues [44,45]; ADNF-9 contains nine amino acid and davunetide (NAP) contains eight amino acid residues [46–48]. It is reported that davunetide protected neurons subjected to oxygen-glucose deprivation at very low concentrations by completely blocked cytochrome *c* release [49]; davunetide could also cross the BBB after intranasal treatment and could be detected in rodents' brains by radio labeled peptides studies [50]; furtherly, intranasal administration davunetide could pass through olfactory nerve into the olfactory bulb, and finally to deep brain and cerebrospinal fluid, and play a role in nutrition and protect the central nervous system. Moreover, daily intranasal administration 1 μ g davunetide significantly improved short-term spatial memory of rats impaired by cholinotoxin AF64A [50], and protected against developmental retardation in apolipoprotein E-deficient mice [51]. Another study found that davunetide reduce neonatal mouse brain cells apoptosis and excitotoxic brain damage [52]. Importantly, davunetide also prevented A β aggregation in vitro experiments [19] and promoted the dissolution of A β plaques in cortical cell and protected neurons against A β neurotoxicity [19]. In the present in vivo study, the neuroprotective effect of davunetide against A β 1–42 was investigated by combination use of behavioral test and electrophysiological recording. In MWM test, we found that bilateral intrahippocampal injection of A β 1–42 significantly impaired spatial learning and memory of rats, with increased escape latency in hide platform test and decreased target quadrant swimming distance in probe trail; while three weeks intranasal administration of davunetide basically reversed these deficits induced by A β 1–42. In hippocampal LTP recording, we found that davunetide did not affect the basic synaptic transmission, but protected against A β 1–42 induced suppression in hippocampal LTP. It is notable that our behavioral tests and electrophysiological recording were performed on the same animals. The consistency of the results from MWM and LTP tests helps us to best understand the close association between spatial memory and synaptic plasticity, and provides more convincing evidence that davunetide might be beneficial in attenuating AD-related deficits in learning and memory.

The PI3k/Akt pathway is an important intracellular signaling pathway in regulating cell survival, proliferation, and synaptic transmission [30]. The Akt kinase (also called protein kinase B) activity is induced following activation of PI3K in growth factor receptor mediated signaling cascades [30,53]. Upon receptor tyrosine kinase (RTK)-dependent activation by growth factors, PI3K complexes are recruited to the plasma membrane. In addition to lipid kinase activity and resulting PIP3 production, PI3Ks also exert serine/threonine protein kinase activity against protein substrates which leading to the phosphorylation and activation of Akt linked to membrane PIP3. Once activated, Akt promotes downstream biological responses. In neuronal cells, activated Akt regulates diverse targets that have been implicated in the regulation of protein translation and cell size, axonal outgrowth, apoptosis suppression and synaptic plasticity. Several lines of in vitro evidence using neuronal cell lines and dispersed primary neuronal cultures have demonstrated a requirement for Akt in the protection against trophic factor deprivation, oxidative stress and ischemic injury [54–56]. Other studies also have

shown that Ras/MAPK and PI3k/Akt kinases mediated anti-apoptotic effect of BDNF against glutamate-induced neuronal death on excitotoxic brain damage model [57]. In support of a direct involvement of Akt in synaptic function, studies directed at working memory performance performed in Akt1 (–/–) mice [58] found a strong correlation between Akt activity and cognitive performance. Additionally, this pathway has been found to be a necessary component in neural long term potentiation.

Interestingly, it has been shown that the PI3k/Akt pathway was related to A β -induced memory impairment in drosophila [59]. Dysregulation of Akt activity was also observed in neurodegenerative diseases including AD [60]. A β 25–35 could reduce Akt activity on PC12 cells. Similarly, we found, in the present study that intrahippocampal injection of A β 1–42 oligomers did not affect the total Akt level, but induced a significant decrease in the level of pAKT, as well as impairments in spatial memory and synaptic plasticity. The PI3k/Akt pathway has also been implicated in the neuroprotective effects of some growth factors, such as BDNF and ADNP. Luckily, we found that chronic intranasal administration of davunetide not only prevented the pAKT decline, but also antagonized A β 1–42 induced memory deficits and LTP suppression. Therefore, we deem that the improvements of spatial memory and synaptic plasticity by davunetide are probably involved in the activation of growth factor receptor mediated PI3k/Akt pathway. Thus, davunetide, or other activator of PI3k/Akt pathway, might be beneficial to the degenerative Alzheimer's disease. In fact, we have pleased to see that davunetide has been used in clinical trials in patients suffering from amnesic mild cognitive impairment, without significant side effect [61–63].

Acknowledgments

This work was supported by National Science Foundation of China (31271201, 31300968, 31471080), Graduate Students Outstanding Innovation Project Foundation of Shanxi Province (20143061) and 331 Early Career Researcher Grant of the Basic Medical School, Shanxi Medical University.

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