



Neuroprotective effects of nootkatone from *Alpiniae oxyphyllae* Fructus against amyloid- β -induced cognitive impairment

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Abstract

The sesquiterpene nootkatone (NKT), isolated from *Alpiniae oxyphyllae* Fructus, was shown to possess protective effects on neurons. In our study, by using an Alzheimer's disease (AD) model of mice induced by intracerebroventricular (i.c.v.) injection of A β_{1-42} oligomers, we investigated the effects of NKT on memory impairment and further evaluated the pathological changes of mice. AD mice were treated by i.c.v. injection of NKT (at a dose of 0.02 mg/kg and 0.20 mg/kg) or vehicle (PBS) into the lateral ventricle once daily for 5 consecutive days. The behavioral tasks were performed, and levels of some biochemical indicators and histopathological changes of the brain were evaluated to elucidate the mechanism of NKT in the treatment of AD. The results revealed that NKT significantly improved the neurobehavioral performance of the AD mice in the Y-maze and Morris water maze tests. More importantly, NKT treatment decreased the malondialdehyde (MDA), A β as well as the acetylcholin esterase (AChE) levels in the mice brain, while increased the glutathione peroxidase (GSH-Px) levels with improved histopathological changes in the hippocampus. These findings provided evidences for the beneficial role of NKT in A β_{1-42} -induced mice AD model linking to anti-oxidative and anti-AChE activities with inhibitory effect against A β accumulation.

Keywords Alzheimer's disease · Amyloid β -peptide (A β) · Nootkatone · Oxidative stress · Acetylcholin esterase (AChE) · Neuroprotective effect

Introduction

Alzheimer's disease (AD) is a progressive and complex neurodegenerative disorder characterized by the occurrence of senile plaques and neurofibrillary tangles (Querfurth and LaFerla 2010; Sierksma et al. 2013). The disease is pathologically characterized by extracellular deposited amyloid β -

peptide (A β) and intracellular hyper-phosphorylated and tangled tau-protein (Butterfield et al. 2001; Ho et al. 2005; Hynd et al. 2004). A β deposition is known to increase oxidative stress, which is arisen from the imbalance between pro-oxidants and antioxidants that leads to excess generation of ROS and free radicals (Rottkamp et al. 2001). Oxidative stress damages the polyunsaturated fatty acids leading to the disruption of cell membrane and its integrity, inactivation of antioxidant enzymes, and neuronal dysfunction and death (Javed et al. 2012). In addition, reduction in cholinergic activity is correlated with the degree of cognitive impairment and is associated with decreased levels of the neurotransmitter acetylcholine (ACh). Acetylcholinesterase (AChE), the enzyme for cholinergic neurons, is involved in the breakdown of ACh and termination of its neurotransmitter action (Ibach and Haen 2004). It was reported that some natural products might slow the progression of AD because they could simultaneously protect neurons from oxidative stress or act as cholinesterases inhibitors (Williams et al. 2011).

Alpiniae oxyphyllae Fructus (Zingiberaceae) is widely cultivated in South China. The fruits of this plant, 'Yizhi'

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in Chinese, have been used as traditional Chinese medicine for the treatment of intestinal and urethral disorders (Chinese Pharmacopoeia Commission 2010). Previous pharmacological studies have indicated that the extracts of this plant also possess protective effects on neurons and preventative effect on dementia (Koo et al. 2004; Kubo et al. 1995; Shi et al. 2014). However, the components responsible for its protective activity of the nervous system are still unclear.

Nootkatone (NKT) (4,4a,5,6,7,8-Hexahydro-6-isopropenyl-4,4a-dimethyl-2(3H)-naphthalenone) (Fig. 1a), one of the major sesquiterpenoid components in *A. oxyphylla*, is widely used for flavoring the food and tobacco (Davies and Deroles 2014). Our preliminary work had found the chloroform extract and n-butanol extract from the fruits of *Alpiniae oxyphyllae* might offer a useful therapeutic choice in either the prevention or the treatment of AD (Shi et al. 2014, 2015). As one of the important bioactive sesquiterpenes of the chloroform extract of *Alpiniae oxyphyllae* Fructus, we investigated the effects of NKT on memory impairment and neurodegeneration in animal models of AD in the current study.

Materials and methods

Animals

Sixty male Kunming mice weighing 30–35 g were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The mice were maintained on standard laboratory conditions of temperature 25 ± 1 °C and a 12-h light/12-h dark cycle with food and water available ad libitum for the duration of the study. The animal study was carried out in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

Drugs and chemicals

$A\beta_{1-42}$ was purchased from Sigma-Aldrich Inc. (St. Louis, MO), and dissolved in PBS (1 mg/ml final concentration) and incubated at 37 °C for 5 days to obtain the oligomers (Maurice et al. 1996). NKT with purity >98% (HPLC) was isolated from the fruits of *Alpiniae oxyphyllae* Fructus, and

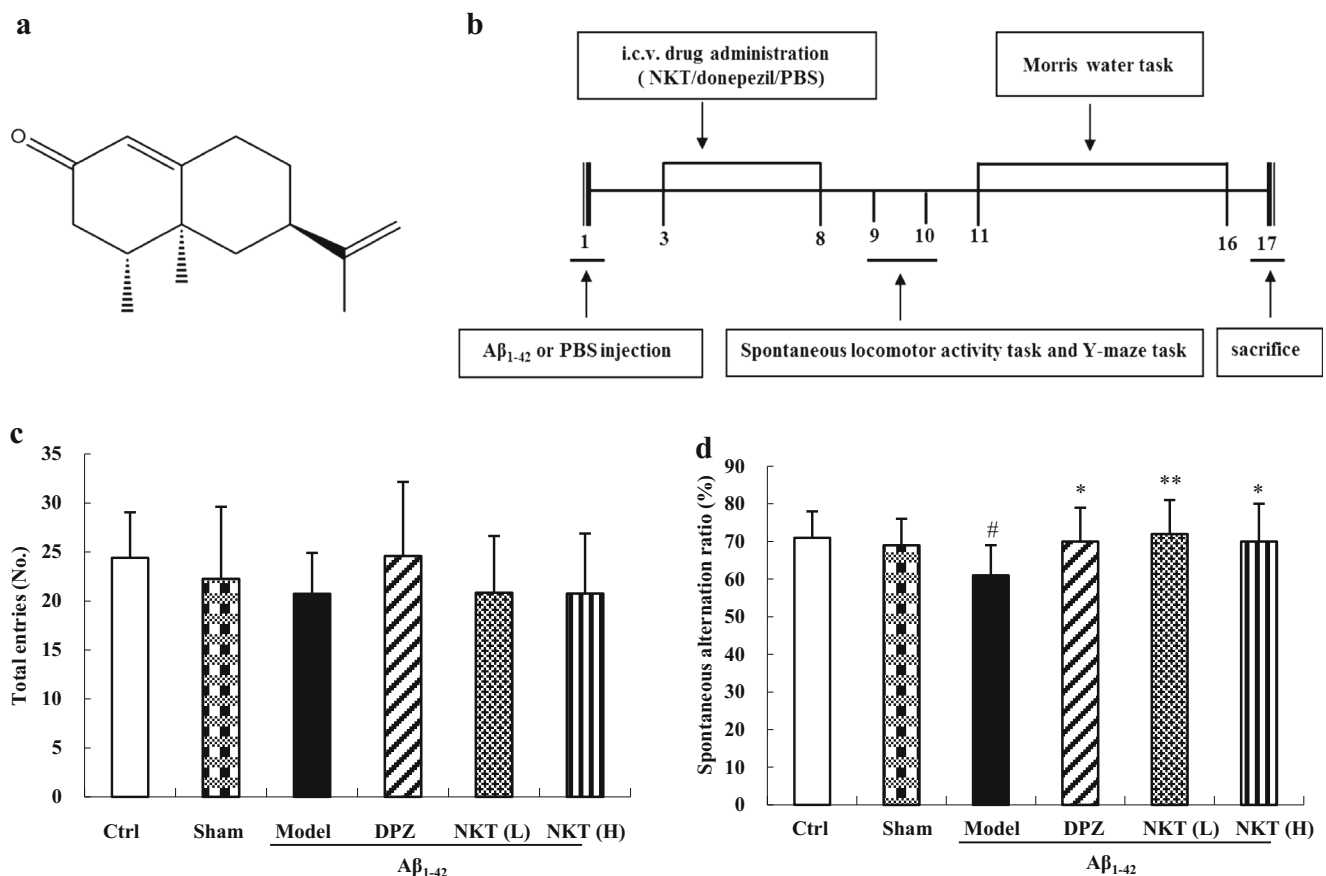


Fig. 1 The structure of NKT (a) and experimental design (b). Effects of NKT on total entries number (c) and spontaneous alternation ratio (d) in the Y-maze task were evaluated. Values are means \pm S.E.M. ($n = 10$

animals per group). [#] $p < 0.01$ v.s. control group; ^{*} $p < 0.05$ and ^{**} $p < 0.01$ v.s. model group

suspended in PBS at a stock concentration of 0.2 mg/ml and 2.0 mg/ml. Donepezil with purity >98% (HPLC) was provided by Melone Pharmaceutical Co., Ltd. (Dalian, China), and suspended in PBS at the concentration of 0.2 mg/ml. Commercial kits for detection of malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GSH-Px), AChE, β -secretase and $A\beta_{1-42}$ were purchased from Nanjing Jiancheng Bio engineering Institute (Nanjing, China).

Experimental design

The mice were divided randomly into 6 groups ($n = 10$ in each group): (i) control group: normal mice without any treatment; (ii) sham group: saline-injection plus intracerebroventricular (i.c.v.) treatment with saline; (iii) model group: $A\beta$ -injection plus i.c.v. treatment with saline; (iv) donepezil (DPZ) group: $A\beta$ -injection plus i.c.v. treatment with donepezil (14 $\mu\text{g}/\text{kg}/\text{day}$); (v) NKT(L) group: $A\beta$ injection plus i.c.v. treatment with NKT (0.02 mg/kg/day); (vi) NKT(H) group: $A\beta$ injection plus i.c.v. treatment with NKT (0.20 mg/kg/day). For $A\beta_{1-42}$ i.c.v. injection, mice were anesthetized by 4% chloral hydrate (10 ml/kg), and $A\beta_{1-42}$ oligomers or vehicle (PBS) was respectively administrated by stereotaxic injections with a Hamilton micro syringe fitted with a 26-gauge needle. Injection (3 μl for each mouse) was administered over 1 min into the dorsal hippocampus at the coordinates -0.5 mm anterior to posterior (AP) bregma, -1.1 mm mid to lateral (ML), -3.0 mm dorsal to ventral (DV) dura. The needle remained in position for an additional 3 min after injection. After $A\beta_{1-42}$ injection, animals were implanted with cannula (10 mm, 23 gauge) located 1 mm above the right ventricle (AP: $+0.2$ mm, ML: $+1.0$ mm, DV: -3.0 mm). The cannula was fixed to the skull with a screw and dental cement. Following surgery, animals were allowed to recover under a heat lamp (Liu et al. 2010). All mice with surgery received daily intraperitoneal injections of 0.2 mL penicillin sodium (200,000 IU/mL) for consecutive 3 days for prevention of infection (Wu et al. 2017). For i.c.v. administration of drugs, NKT, DPZ or physiological saline was given to animals in each group once daily for 5 consecutive days, mice in sham-operated and model groups received same volume of physiological saline i.c.v. injection. The experimental design was shown in Fig. 1b.

Behavioral analyses

Spontaneous locomotor activity task Spontaneous locomotor activity was determined in mice that were placed in a transparent chamber (25 cm diameter \times 13 cm height) connected to an automatic registration system. The chamber was equipped with nine infrared sensors arranged along the bottom of the wall of the arena. The interruption of beams of two consecutive infrared sensors was collected for 5 min as a reflection of locomotor activity. After each testing session, the enclosures were thoroughly cleaned with 70% ethanol and water (Nagakannan et al. 2012).

Y-maze task The Y-maze test involves both cognitive and activities/exploratory behavioral components. The maze is made of black Plexiglas with three identical arms: each arm is 30 cm long, 12 cm high, 5 cm wide at the bottom, and 10 cm wide at the top. Each mouse was placed at the center of the apparatus, and allowed to move freely through the maze during a 6 min period. The number of arm entries was recorded visually. Alternation was defined as successive entry into the three arms, on overlapping triplet sets. Alternation rate (%) was calculated as the number of actual alternations performed divided by the number of possible alternations (defined as the number of arm entries minus two) multiplied by 100 (He et al. 2013).

Morris water maze task Spatial learning and memory retention were tested in the standard Morris water maze protocol. The maze is a circular pool with 150 cm in diameter made of plastic and painted flat black on the interior walls and floor. The maze is surrounded on all sides by curtains to avoid visual interference during trial. Each wall had unique, large geometric figures mounted on them. The pool was filled to a depth of 80 cm with water made opaque by the addition of a nontoxic paint and temperature was maintained between 23 and 27 °C. The goal quadrant contained a clear, 9 cm diameter submerge platform submerged 1 cm below the water. Mice were trained for 5 days including 2 trials daily with a trial interval of 30 min to find the hidden escape platform. Mice were randomly placed into the pool at one of two starting locations. Mice were guided to the platform when they failed to find the platform within 90 s. After 15 s, they were returned to their cages. The time in which mice reached the platform (escape latency in seconds) was evaluated. For memory retention test on the 6th day, the platform was removed and the animals were allowed to swim for 90 s. The time spent in the target quadrant and the number crossed the platform were recorded in memory retention test.

Biochemical and histopathological tests

Biochemical analysis Mice were deeply anesthetized after behavioral tests, the cerebral cortex and hippocampus of 8 mice in each group were dissected out and immediately stored at -80 °C. For biochemical analysis, the cerebral cortex and the hippocampus tissue were homogenized in ice-cold saline and centrifuged at 2000 rpm for 10 min, and the supernatant was collected. Levels of SOD, GSH-px, GSH-Px, β -secretase, AChE, $A\beta_{1-42}$ and MDA were measured using commercial assay kits according to the manufacturers' protocols.

Histological staining Two mice in each group were perfused transcardially with saline followed by 4% paraformaldehyde (PFA) in phosphate buffer saline (0.1 mol/l PBS, pH 7.2) after anesthesia. The whole brains of mice were removed and

postfixed in 4% PFA at 4 °C until sectioned. Coronal sections of the hemisphere were stained with hematoxylin and eosin (H&E) staining. The sections were deparaffinized and washed with PBS, then stained with hematoxylin for 5 min. After washed with tap water, sections were differentiated in acid alcohol and washed again with tap water. Followed by staining with eosin for 5 min, sections were dehydrated with alcohol and mounted.

Statistics analysis

Data were expressed as the mean \pm SEM. The statistical significance in the behavioral and biochemical effects of NKT was determined using SPSS 17.0 (IBM Corp., New York, NY) where statistical significance were assessed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, $p < 0.05$ was considered statistically significant.

Results

NKT improved short-term memory without affecting spontaneous locomotor activity of mice injected with A β_{1-42} peptide

As shown in Table 1, there was no significant difference in the spontaneous locomotor activity indicated by exploratory behavior among different groups ($p > 0.05$). The results suggested that the surgeries or i.c.v. injection of A β_{1-42} or NKT had no effect on spontaneous locomotor activity of mice.

Accordingly, there was no significant difference among groups in the number of total entries in the Y-maze task ($p > 0.05$), which further indicated that surgeries or i.c.v. injection of A β_{1-42} or NKT failed to affect locomotor activity (Fig. 1c). However, the model group showed lower level of the alternation ratio compared with the control group

($p < 0.01$), indicating the short-term memory was impaired by A β_{1-42} . Moreover, in comparison with model group, DPZ, NKT(L) and NKT (H) treatment group showed higher levels of alternation ratio ($p < 0.05$), which indicated an improvement of A β_{1-42} -induced short-term memory impairment in mice by NKT in the Y-maze task. In addition, there was no significant difference between the control group and the sham-operated group ($p > 0.05$) (Fig. 1d).

NKT improved spatial learning and retention memory impairments induced by A β_{1-42} peptide

Acquisition training of the Morris water maze task was carried out for mice to learn to find the hidden platform. Changes of escape latency in the training trials were shown in Fig. 2a. Compared with the control group, model mice exhibited prolonged escape latency during the 5 days training in the place navigation test ($p < 0.01$). Four days after training, both low and high dose of NKT-treated mice required less time to find the hidden platform ($p < 0.05$). Similarly, DPZ group exhibited a significant decrease of escape latency compared with model mice on the last day of training ($p < 0.05$).

Memory retention of the platform location was assessed in a 90 s spatial probe test performed on the day following the place navigation test. Figure 2b showed that model mice spent significantly less time in the target quadrant compared to control mice ($p < 0.01$), while NKT or DPZ treatment restored the time spent in the target quadrant in mice injected with A β_{1-42} . Accordingly, the number of crossing the platform site of mice in model group was lower than those in control group ($p < 0.01$), while mice treated with NKT or DPZ crossed the platform site significantly more times than the model mice ($p < 0.05$) (Fig. 2c). In the place navigation test and spatial probe test, there was no significant difference between the control group and the sham-operated group ($p > 0.05$) (Fig. 2a–c).

NKT treatment improved hippocampal neuronal damage in mice with A β_{1-42} injection

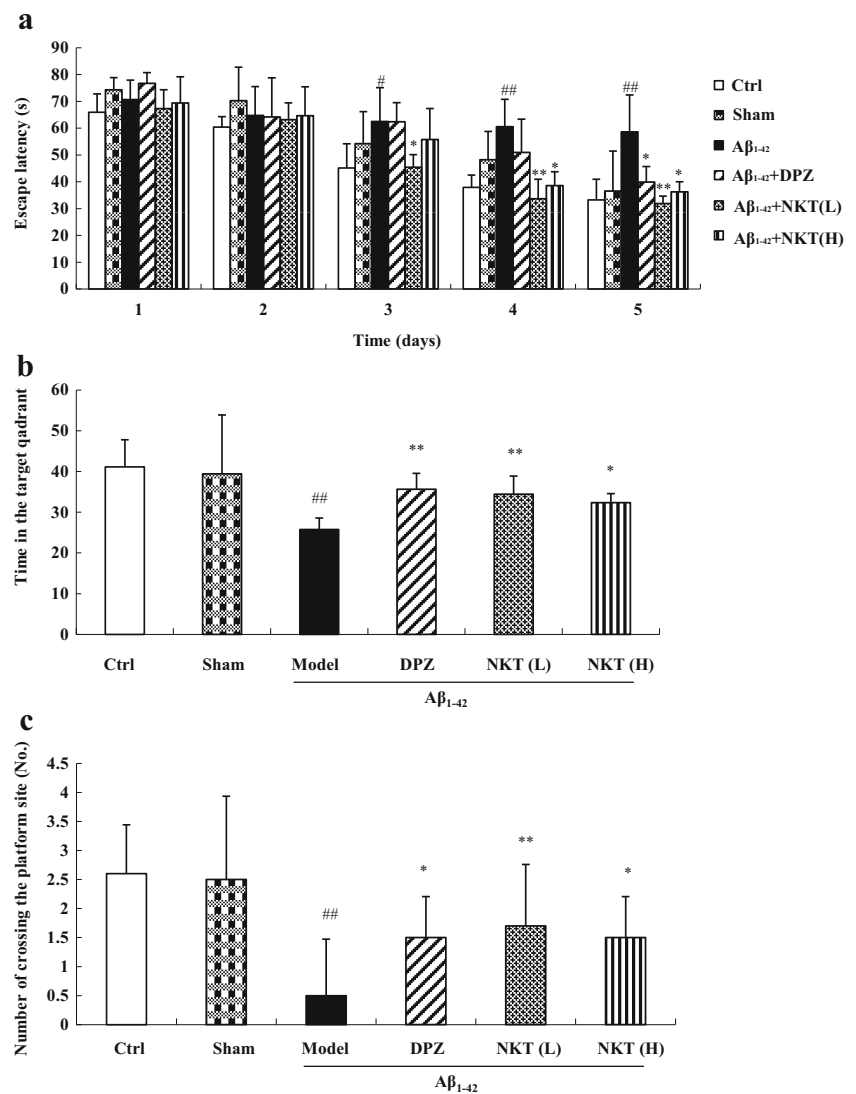
H&E staining was performed to detect the neuronal integrity and orderliness, as well as cell morphology and tissue structure in hippocampal CA1 region. The neurons in control and sham-operated groups appeared to be intact and ordered (Fig. 3a, b), however, eccentrically dispersed nuclei and disordered arrangement of neurons were found in the model group (Fig. 3c). The histopathological changes were significantly ameliorated by DPZ treatment (Fig. 3d). Similarly, NKT (0.02 mg/kg and 0.20 mg/kg) significantly increased the number of neurons, and the degree of the neuronal injury was decreased in mice with A β_{1-42} injection (Fig. 3e, f).

Table 1 Effects of NKT on number of activities and activity time in the spontaneous locomotor activity task

Groups	Number of activities	Activity time (s)
Control	21.9 \pm 11.5	230.4 \pm 20.3
Sham-operated	19.9 \pm 11.1	231.1 \pm 27.9
Model	21.0 \pm 10.1	242.9 \pm 16.1
Donepezil	20.6 \pm 9.0	232.1 \pm 28.3
NKT(0.02)	19.8 \pm 8.6	230.4 \pm 37.9
NKT(0.20)	20.7 \pm 6.9	246.2 \pm 17.3

Values are means \pm SEM. (n = 10 animals per group)

Fig. 2 Effects of NKT on escape latency (a), time in the target quadrant (b) and the number of crossing the platform site (c) in the Morris water maze test. Values are means \pm S.E.M. (n = 10 animals per group). $^{###}p < 0.01$ v.s. control group; $^{*}p < 0.05$ and $^{**}p < 0.01$ v.s. model group



NKT decreased MDA level and increased GSH and GSH-Px levels in brain of mice with A β_{1-42} injection

The MDA level in the frontal cortex of model mice was significantly higher than the control mice ($p < 0.01$). This increase was attenuated by treatment of NKT at doses of 0.02 and 0.20 mg/kg ($p < 0.01$). However, there was no significant difference in the hippocampus among the groups, although the model group showed the obvious trend of increasing MDA level ($p > 0.05$) (Table 2).

The level of GSH decreased remarkably both in the frontal cortex and hippocampus of model mice compared with the control mice ($p < 0.01$). NKT at the both doses could reverse the GSH level in the frontal cortex ($p < 0.01$), while the treatment failed to up-regulate the GSH level in the hippocampus ($p > 0.05$) (Table 2).

Table 2 also showed a significant decrease in GSH-Px level in the frontal cortex and hippocampus of

model mice as compared with the control group ($p < 0.01$). DPZ and both doses of NKT significantly increased GSH-Px level in hippocampus of mice with A β_{1-42} injection ($p < 0.05$).

NKT showed inhibitory effect on accumulation of A β in the hippocampus without affecting the level of β -secretase in the cortex

As shown in Fig. 4a, an increased accumulation of extracellular A β in the hippocampus of A β_{1-42} -injected mice was observed compared with the control mice ($p < 0.05$). However, NKT prevented the accumulation of A β in the mice received A β_{1-42} injection ($p < 0.05$). The level of β -secretase in the cortex of A β -injected mice brain was further analyzed (Fig. 4b). In comparison with the control mice, the β -secretase level of the model mice was significantly higher ($p < 0.05$), while it was not further affected by NKT treatment ($p > 0.05$) (Fig. 4b).

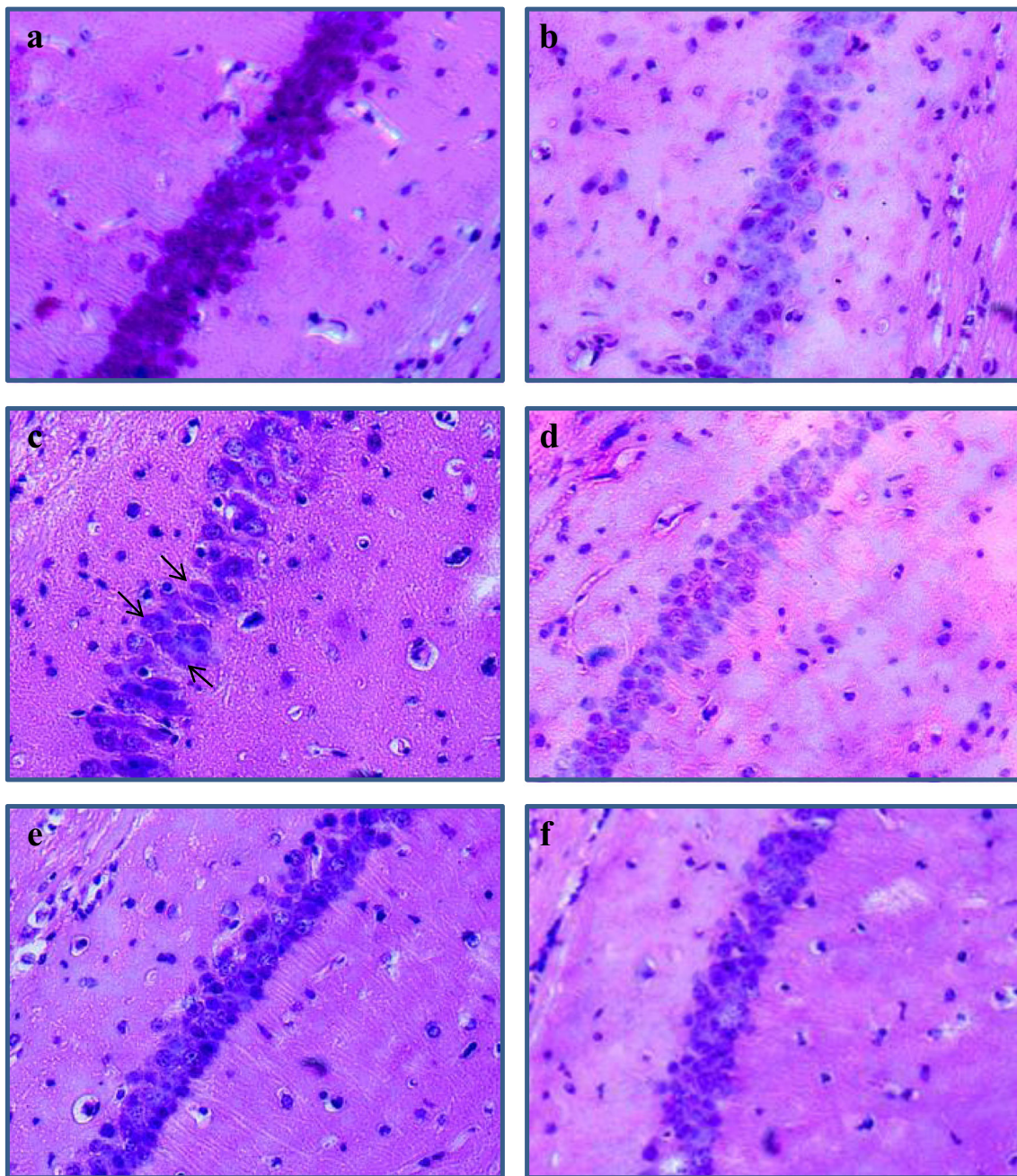


Fig. 3 Effects of NKT on the morphology and the number of neurons in an A β -induced AD mouse model. Light micrographs of hippocampal neurons from the CA1 region of control group (a), sham-operated group

(b), model group (c), donepezil group (d) and NKT at doses of 0.02 mg/kg group (e) and 0.20 mg/kg group (f). The arrows indicate the neurons with degenerated nuclei

NKT inhibited AChE activity in brain of mice with A β_{1-42} injection

The activity of AChE within the cortex and hippocampus were increased in model mice with A β_{1-42} injection ($p < 0.01$) (Fig. 4c, d). The administration of DPZ or both high and low doses of NKT significantly down-regulated the activity of AChE in cortex ($p < 0.01$) (Fig. 4c). The increased activity of AChE was also

significantly inhibited by the treatment with NKT in the hippocampus ($p < 0.01$) (Fig. 4d).

Discussion

Alzheimer's disease (AD) is a neurodegenerative disorder of advanced age characterized by loss of memory and the accumulation of A β deposits and decreased levels of the

Table 2 Effects of NKT on MDA, GSH and GSH-Px levels in the frontal cortex and hippocampus of mice with A β_{1-42} injection

Group	Frontal cortex			Hippocampus		
	MDA (nmol/mg)	GSH (μ mol/mg)	GSH-Px (U/mg)	MDA (nmol/mg)	GSH (μ mol/mg)	GSH-Px (U/mg)
Control	4.08 \pm 1.18	259.7 \pm 27.60	242.5 \pm 19.16	1.51 \pm 0.37	261.8 \pm 24.10	253.2 \pm 33.58
Sham-operated	6.21 \pm 0.37	263.9 \pm 35.21	249.8 \pm 30.20	1.38 \pm 0.45	251.7 \pm 26.30	246.5 \pm 14.80
Model	12.39 \pm 2.10 ^{##}	136.8 \pm 40.08 ^{##}	122.4 \pm 31.97 ^{##}	1.97 \pm 0.92	118.2 \pm 20.42 ^{##}	130.1 \pm 19.62 ^{##}
Donepezil	9.68 \pm 4.68	156.3 \pm 23.97	209.7 \pm 35.55*	1.54 \pm 0.41	156.1 \pm 23.99	184.0 \pm 29.39*
NKT(L)	3.67 \pm 0.63**	240.4 \pm 14.61**	215.6 \pm 23.07*	1.39 \pm 0.38	141.7 \pm 42.97	239.0 \pm 23.71**
NKT(H)	4.01 \pm 1.62**	229.8 \pm 19.05**	127.5 \pm 29.28	1.28 \pm 0.29	141.5 \pm 41.32	181.1 \pm 38.91*

Data are shown as mean \pm SEM (n = 8 animals per group). ^{##}p < 0.01 v.s. control group; *p < 0.05 and **p < 0.01 v.s. model group

neurotransmitter acetylcholine (Tesseur et al. 2013). DPZ, a selective inhibitor of AChE that had been approved for treatment of AD (Wilkinson 1991), was used as a positive control drug to evaluate the effectiveness of NKT in AD treatment. Because hippocampal dysfunction caused by A β is one of the pathologic mechanisms of AD (Butterfield et al. 2001), we

chose the human A β_{1-42} i.c.v. injection model to test the efficacy of NKT on recovery of memory deficit. Blood-brain barrier (BBB) is a main obstacle to the effective delivery of drugs to the brain (Mao et al. 2015). It is reported that after volatile oil of *Alpiniae oxyphyllae* Fructus was intragastric administrated to rats, nootkatone could be detected in the

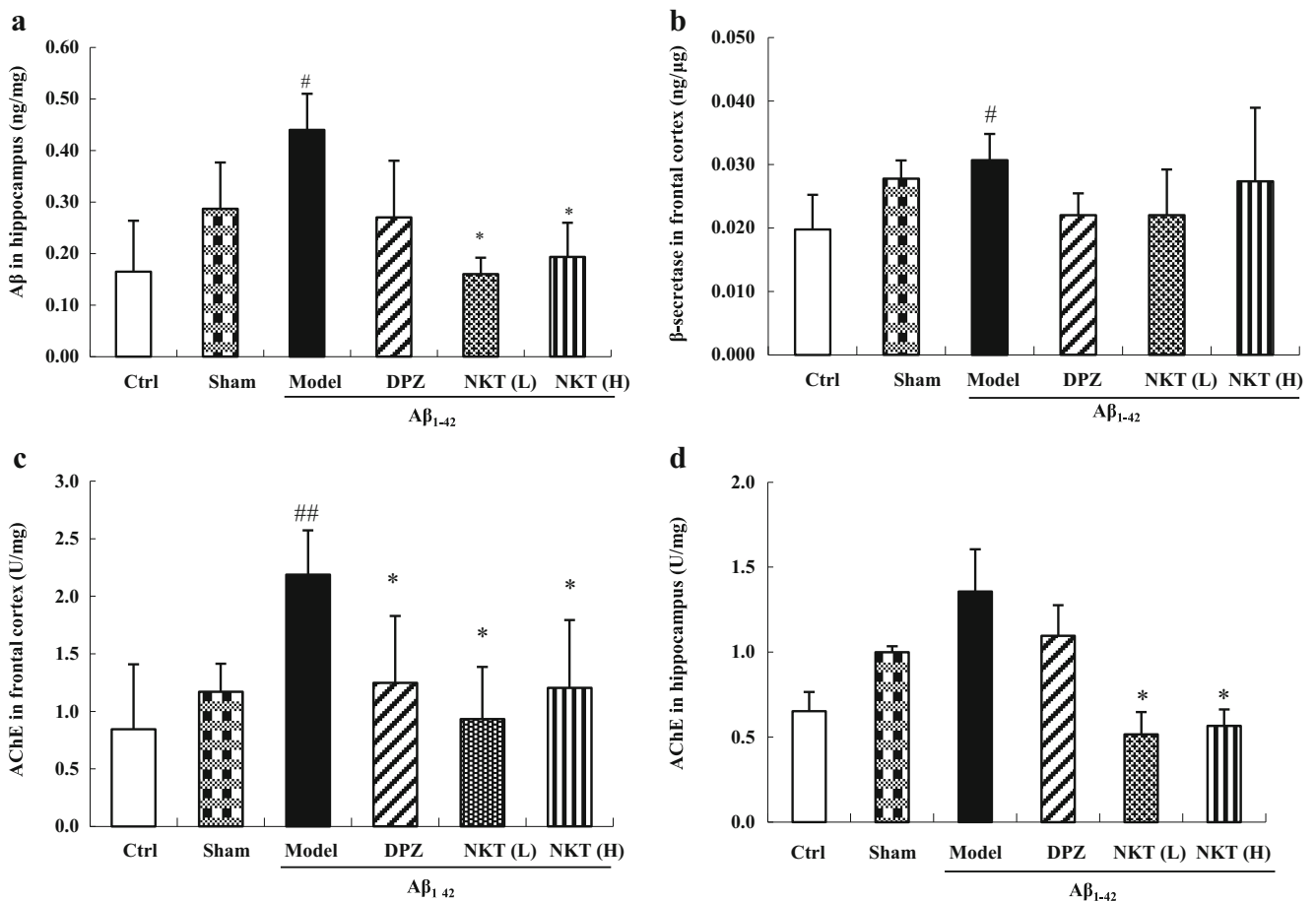


Fig. 4 Effects of NKT on A β accumulation in the hippocampus (a), β -secretase level in the frontal cortex (b), and AChE activity in the cortex (c) and hippocampus (d) of A β_{1-42} -injected mice. Data are shown as mean \pm

S.E.M (n = 8 animals per group). [#]p < 0.05 v.s. control group; *p < 0.05 and **p < 0.01 v.s. model group

cerebrospinal fluid (Tan et al. 2004). The results indicated NKT might cross BBB freely. In the current study, NKT and DPZ were given to mice by i.c.v. injection through an alloy-steel tube to investigate the effectiveness against $A\beta_{1-42}$ induced AD. Our results revealed that $A\beta_{1-42}$ induced marked deficiencies in both short- and long-term memory of mice, and increased deposition and dissemination of $A\beta$ in the hippocampus of mice, which were consistent with the biochemical and histological observations.

Y-maze task was conducted to investigate short term stressless memory processes, while Morris water maze test was performed to evaluate long term stressful learning of mice. These intensive behavioral evaluations allow a detailed description of the non-cognitive behavioral abnormalities and cognitive impairments in this AD model and provide useful information for studies investigating potential treatments or interventions (Mao et al. 2015; Wu et al. 2017). In our research, i.c.v. injection of $A\beta_{1-42}$ impaired both short term and long term memory of mice, while DPZ or NKT treatment could effectively reverse $A\beta_{1-42}$ -induced impairment (Figs. 1 and 2).

All multifactorial pathological conditions of AD are evidenced to link with oxidative stress, which results from imbalance between production and removal of reactive oxygen species (ROS) and leads to neuronal injury (Markesbery 1997). MDA, a by-product of lipid peroxidation, is a major biomarker of ROS accumulation induced membrane lipid peroxidation (Ziech et al. 2010). In the present study, MDA level in the frontal cortex was increased by $A\beta_{1-42}$ induction, which was effectively inhibited by NKT treatment. The level of GSH, an endogenous antioxidant against free radicals (Kharrazi et al. 2008), was decreased in the frontal cortex of model mice, which was restored by NKT i.c.v. injection. Moreover, GSH-Px, functions to reduce lipid hydroperoxides (Kharrazi et al. 2008), was also up-regulated by NKT in mice with $A\beta_{1-42}$ injection (Table 2). Frontal cortex is a cortical region known to be involved in AD, increases in oxidative markers and declines in antioxidants in frontal cortex were AD-dependent (Ansari and Scheff 2010). Our findings indicated an anti-oxidative activity of NKT against $A\beta_{1-42}$ might be involved in the neuroprotective effect. However, DPZ only showed a weak anti-oxidative effect against $A\beta_{1-42}$ (Table 2).

Cholinergic dysfunction and oxidative stresses have been linked to the pathological development of AD (Ho et al. 2003). It has been reported that a reduction in the acetylcholine concentration in the cholinergic synaptic cleft was observed in the AD brain (Craig et al. 2011). As the enzyme responsible for terminating cholinergic neurotransmission, AChE is able to promote $A\beta$ aggregation in neurons, and $A\beta$ could also in turn increase the activity of AChE (Hu et al. 2004). Previous study reported that NKT and some sesquiterpenoids isolated from the ethanolic extract of *Alpinia oxyphylla* Fructus showed weak to moderate AChE inhibitory activities (Chen and Xiang 2013).

Accordingly, our study revealed that the activity of AChE within the cortex and hippocampus were increased in model mice with $A\beta_{1-42}$ injection, while NKT and DPZ significantly down-regulated the activity of AChE in brain tissues. Unexpectedly, AChE was slightly, although insignificantly, activated in sham group compared with control group, which might be caused by the slight inflammation induced by surgery (Fig. 4). Our study indicated that NKT may mediate neuroprotective effect against $A\beta$ -induced cognitive impairment partly through the inhibition of AChE.

Moreover, the accumulation of the $A\beta$ in the brain has been thought to be a key factor in the pathogenesis of AD, and the production of $A\beta$ is due to sequential cleavages of amyloid precursor protein (APP) by β - and γ -secretases (Chami et al. 2012). Levels of $A\beta$ and β -secretase in brain of mice were increased significantly in mice with $A\beta_{1-42}$ injection, although a slightly increase of $A\beta$ and β -secretase was also shown in mice with sham surgery, the difference was not significant. Moreover, our results showed an inhibitory effect of NKT rather than DPZ on the augmentation of $A\beta$ accumulation in hippocampus of brain, while the level of β -secretase was not affected by NKT (Fig. 4). Exogenous $A\beta$ was injected for induction of AD animal model in our study, and results indicated that NKT may directly inhibit accumulation of exogenous $A\beta$ in brain without affecting the activity of β -secretase that promotes endogenous $A\beta$ production. In addition, accordant with the results from biochemical analysis, H&E staining showed that the number of neurons in the NKT-treated mice was significantly increased, the degree of neuronal injury was decreased compared with the model mice (Fig. 3).

In summary, NKT from *Alpinia oxyphylla* Miq. significantly protected mice from $A\beta_{1-42}$ -induced memory impairment in behavioral tests including Y-maze and MWM tasks. The memory ameliorating effects of NKT were mediated, in part, by the anti-oxidative and anti-AChE activities. Additionally, NKT effectively inhibited $A\beta$ accumulation in hippocampus of mice after $A\beta_{1-42}$ injection. Unlike the commercial drugs such as DPZ with single target against AChE, our results suggest that NKT may represent a potential therapeutic to improve cognitive function in patients with AD through the regulation of multi-targets.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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