Protective Effect of Notoginsenoside R1 on an APP/PS1 Mouse Model of Alzheimer's Disease by Up-Regulating Insulin Degrading Enzyme and Inhibiting Aβ Accumulation

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Abstract: Notoginsenoside R1 (NTR1) is the main active ingredient of the well-known traditional Chinese herbal medicine Panax notoginseng, the root of Panax notoginseng (Burk.) F. H. Chen. Studies demonstrated that NTR1 may have some neuronal protective effects. Alzheimer’s disease (AD) is a neurodegenerative disease characterized by β-amyloid protein (Aβ) deposition, neurofibrillary tangle formation and neuronal loss. This study was designed to explore the protective effect of NTR1 on an APP/PS1 double-transgenic mouse model of AD and investigate the possible mechanism. The 3-month-old mice were fed with 5 mg/(kg•d), 25 mg/(kg•d) NTR1 or vehicle via oral gavage for 3 months and changes in behavior, neuropathology, and amyloid pathology were investigated. The mice with NTR1 treatment showed significant amelioration in the cognitive function and increased choline acetyl transferase expression, as compared to the vehicle treated mice. NTR1 treatment inhibited Aβ accumulation and increased insulin degrading enzyme expression in both APP/PS1 mice and N2A-APP695sw cells, suggesting that of NTR1 may exert its protective effects through the enhancement of the Aβ degradation. Furthermore, our data showed that the increased level of peroxisome proliferator-activated receptor γ (PPARγ) and the up-regulation of insulin degrading enzyme induced by NTR1 were inhibited by administration of GW9662 (a PPARγ antagonist), indicating that the effect of NTR1 was mediated, at least in part, by PPARγ. Thus, our findings provide the evidences that NTR1 has protective effect on AD mouse model and NTR1 may be a potential candidate for AD treatment.

Keywords: Notoginsenoside R1, Alzheimer’s disease, insulin degrading enzyme, β-amyloid protein.

1. INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia characterized by β-amyloid protein (Aβ) deposition, neurofibrillary tangle formation and neuronal loss [1]. China has the largest AD patient population in the world, which was 5.69 million in 2010 [2]. AD is becoming a major public health problem and it is urgent to find new effective medication to treat AD.

The accumulation of toxic Aβ is widely considered as a main causative agent of AD. Increased Aβ levels were found in the brains of AD patients and the AD mouse model. Aβ accumulation could cause multiple neuronal damages and result in the cognitive impairments. For instance, it contributes to neuronal and synaptic malfunction by initiating oxidative stress, inflammation and apoptosis [3]. Moreover, it could directly induce cholinergic dysfunction by inhibiting the activity of choline acetyltransferase (ChAT) [4], which is critical for acetylcholine synthesis. Thus, reducing Aβ accumulation may be a promising strategy to improve cognitive function and halt AD progression [5].

Aβ is generated from amyloid precursor protein (APP) via two-step proteolytic processing by β- and γ-secretases [6] and is degraded by catabolic enzymes such as insulin degrading enzyme (IDE) [7]. IDE, a highly conserved Zn (2+)-dependent endopeptidase which is known to degrade insulin, is also important for the steady-state concentration of Aβ by affecting its dynamic equilibrium. IDE deficient mice demonstrated increased cerebral accumulation of Aβ, which suggested that IDE deficiency may contribute to some forms of AD [8]. Therefore, IDE is becoming a new target for preventing Aβ accumulation and AD development.

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herbal medicine which has been widely used for diseases related to the circulatory system, such as cardio- and cerebro-vascular disorders, and liver dysfunction [9-11]. Panax notoginseng has more than 30 different saponins called Panax Notoginsenosidum (PNS). It was reported that Panax notoginseng has protective effects for cholinergic neurons showing the increased level of ChAT and the increased number of cholinergic neurons [12]. Recent studies suggest that PNS has some beneficial effects on AD, such as improving the cognitive function in some chemical induced memory impairment mouse model and reducing the Aβ level in SAMP8 mouse [13-15], but the mechanism needs to be further investigated. Lei et al. have reported the extracts of Panax ginseng and Panax notoginseng significantly increased peroxisome proliferator-activated receptor γ (PPARγ) mRNA and protein expression [16].

Notoginsenoside R1 (NTR1) is the unique and main active ingredient of Panax notoginseng [17]. NTR1 is one of the eminent members of PNS and may have potential protective effects on AD. Pharmacological research suggests that NTR1 has many beneficial effects including the amelioration of microcirculation disturbances as well as the anti-inflammatory and anti-oxidative effects [18-23]. It was reported that NTR1 could decrease tumor necrosis factor-α induced reactive oxygen species production by inhibiting the activation of ERK- NADPH oxidases pathway [20]. It was also found that NTR1 had protective roles in cardiac dysfunction of endotoxemic mice induced by lipopolysaccharide via activation of oestrogen receptor α and PI3K/PKB (Akt) signaling [19]. Recent studies showed that NTR1 had neuroprotective effects in the H2O2 stimulated PC12 cells and neuroprotection against the cerebral ischemia-reperfusion injury in vivo and in vitro by inducing estrogen receptor-dependent activation of Akt/Nrf2 pathways and suppressing the oxidative impairments [24, 25]. Further, NTR1 could protect the cells by attenuating the oxidative damage induced by Aβ in neurons and restoring mitochondrial membrane potential [26]. Moreover, our recent study has shown that NTR1 can increase the neuronal excitability and reverse Aβ oligomers-induced long term potentiation impairments in the acute hippocampal slices [27]. However, whether NTR1 could regulate the metabolism of Aβ is still unknown. As mentioned above, IDE participates in the proteolysis of Aβ. PPARγ regulates IDE expression by binding to a peroxisome proliferator-response element (PPRE) in the IDE promoter [28]. Therefore, we hypothesize that NTR1 increases IDE expression by up-regulating PPARγ expression, and as a result, the Aβ levels are decreased in the brains; at the same time, NTR1 increases the ChAT expression and the number of cholinergic neurons, which can also have beneficial effects in AD patients. To explore the potential anti-AD effect of NTR1, evaluate our hypothesis and provide a new candidate for the treatment of AD, we investigated the protective effect of NTR1 on an APP/PS1 double transgenic mouse model of AD and an APP695sw overexpressing N2a cell line.

2. MATERIAL AND METHODS

2.1. Reagents

The NTR1 (Fig. 1) was purchased from Nanjing Zelang Medical Technology (Nanjing, Jiangsu, China, >98%, ZL20111215S). NTR1 was dissolved in sterile distilled water before use. GW9662 and DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture medium, GlutaMax supplement and penicillin & streptomycin were purchased from Life technologies (Grand Island, NY, USA). G418 was ordered from Amresco (solon, Ohio, USA). Fetal bovine serum was purchased from PAA laboratories (Pasching, Austria). Primary antibodies used in this study were ordered as follows: 6E10 (Covance, Princeton, NJ, USA), ChAT, GAPDH and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), IDE (Abcam, Cambridge, MA, USA), PPARγ (Cell signal technology, Danvers, MA, USA).

![Fig. (1). Structure of Notoginsenoside R1.](image)

2.2. Animals and Grouping

3-month-old male APP695sw/PS1ΔE9 C57BL/6J (APP/PS1) mice and C57BL/6J (C57) mice were purchased from the Institute of Laboratory Animal Sciences, CAMS & PUMC (Beijing, China). The animals were housed in a standard specific-pathogen-free facility (25 ± 2 °C, 40–60% humidity, and 12 h light/dark cycle) with free access to water and regular rodent diet. The APP/PS1 mice were randomly divided into 3 groups (n=10 mice per group), which received the low dose of NTR1 (NTR1-low, 5 mg/(kg•d)), the high dose of NTR1 (NTR1-high, 25 mg/(kg•d)), or the vehicle (sterile distilled water) respectively via oral gavage. Age-matched C57 mice (n=10) were treated with the vehicle (sterile distilled water) and used as a normal control group. After 3 months of NTR1 administration, the Morris water maze task was conducted to evaluate the memory function of the mice. Then the animals were euthanized, and various biochemical and histological parameters were evaluated.
2.3 Behavioral Studies

The spatial learning and memory of the mice were evaluated using the Morris water maze [29]. Large, high-contrast geometrical patterns were mounted on the walls of the room to serve as distant spatial landmarks that the mice learned to recognize as it formed a cognitive map of the environment. The environments and experimenter were unchanged throughout the experiment. In the hidden platform test, a platform was placed at the center of one suppositious quadrant and submerged 1 cm below the water level. The mice were administered four trials per day for five consecutive days (day 1-5). During each trial, the mice were released from four semi-randomly assigned starting points and allowed to swim for 90 s. After mounting the platform, the escape latency was recorded by software. If a mouse failed to reach the platform within 90s, it was guided to the platform. In both situations, the animals were allowed to rest on the platform for 15 s and then placed in the home cage. On the day after the hidden platform test, probe trials were performed (day 6) with the platform removed. The animal was released from the opposite quadrant and allowed to swim freely for 60 s. On the visible-platform test (day 7), the platform was elevated above the water surface and placed in a different position. The mice were given four trials. All of the experiments were conducted at approximately the same time each day.

2.4. Cell culture

The N2A-APP695sw cell line that stably expresses Swedish mutant human APP695 is constructed and stored by Beijing Key Laboratory for the Protection and Utilization of Traditional Chinese Medicine Resources of Beijing Normal University. N2A-APP695sw cells were maintained in DMEM/Opti-MEM (1:1, v/v), with 500 µg/ml G418, 5% fetal bovine serum, 100 units/ml penicillin, 100 mg/ml streptomycin, 1% GlutaMax, and kept at 37 °C in humidified 5% CO2/95% air. The cells were passaged every 3 days. The cells were planted on 6 or 12 well plates at the density of 5×10⁵/ml. After the confluence reached 80%, the cells were treated with varying doses of NTR1 (1-100 µM) for the indicated time. Cells were treated with 20 µM GW9662, a PPARγ antagonist, for 1 h before the co-incubation with NTR1. The cells were harvested and lysed for western blotting. The conditioned medium was collected for Aβ ELISA.

2.5. Aβ ELISA Assay

The Human Aβ40 ELISA Kit and the Human Aβ42 ELISA Kit (Life technologies) were used for Aβ determination. For tissue samples, about 10 mg of cortex or hippocampus tissue was homogenized in eight times the volume of ice-cold buffer A (5 M guanidine hydrochloride, 50 mM Tris-HCl, pH 8.0) and lysed in RT for 4 h. Samples were then centrifuged at 16000×g for 20 min in 4 °C and the supernatant was collected. For cell samples, the conditioned media were collected and followed by concentrating using uliracel-3 K centrifugal filters (Millipore IRELAND, Carrigtwohill, CO.CORK, IRL). The protein concentrations of the tissue lysate and the condensed media were determined by the BCA method. The Aβ40 and Aβ42 concentrations of the samples were then determined following the manufacturer’s protocol. Optical densities at 450 nm of each well were read on a Multiskan MK3 microplate reader (Thermo Labsystems, Waltham, MA, USA), and Aβ40 and Aβ42 concentrations were determined by comparison with the Aβ40 and Aβ42 standard curves. All readings were in the linear range of the assay.

2.6. Immunohistochemistry Analysis

The level of Aβ plaques, ChAT and IDE in the mice brain were determined by immunohistochemistry (IHC). After perfusion with saline, the mice brains were separated and divided sagittally in half. One half was immersed in paraformaldehyde (4%) overnight, followed by dehydration and embedding. The paraffin sections (10 µm) were cut and deparaffinized. The sections were pre-incubated in a boiling sodium citrate buffer (pH 6.0) for the antigen retrieval, and the blocking of endogenous peroxidase non-specific binding sites activity was performed according to the standard protocols. The primary antibodies against Aβ (6E10) (1:100), ChAT (1:50) or IDE (1:50) were incubated with the sections at 4 °C overnight. Subsequently, a Streptavidin/Peroxidase stain kit and DAB kit (Life Technologies) were used to produce a brown color. The specificity of the labeling was established by omitting the primary antibody. Image J software (National Institutes of Health, Bethesda, MD, USA) was used for the image analysis.

2.7. Western Blotting

The cerebral cortex was carefully dissected and immediately frozen in liquid nitrogen. Approximately 20 mg of tissue was lysed in 10 volumes (wt/vol) of RIPA buffer containing protease inhibitors (Applygen, Beijing, China) and centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was reserved, and the protein concentrations were determined by the BCA method. The protein concentrations of samples were adjusted to equal level by using lysis buffer. The adjusted samples were then mixed with 2×loading buffer equivalently and boiled for 5 min. For the cell samples, the cells were rinsed twice with PBS after the medium was removed. The cells were lysed in 1×loading buffer and boiled for 5 min. The samples were loaded into SDS-PAGE and blotted (295 mA, 1.5 h). After blocking with 5% fat-free milk powder, the primary antibodies against ChAT (1:200), APP (6E10) (1:250), IDE (1:400), β-actin (1:5000), PPARγ (1:500), and GAPDH (1:200) were used. GAPDH or β-Actin was used as a loading control for general protein contents. The source matched secondary antibodies (LI-COR, INC. Lincoln, NE, USA) were used and the membranes were scanned by Odyssey 9120 (LI-COR, INC.). Bands were analyzed by Odyssey software (LI-COR, INC.).

2.8. Reverse Transcription-PCR

The total RNA of cortex tissue was extracted using RANprep pure Tissue Kit (TIANGEN, Beijing, China). The
APP/PS1 mice showed impaired cognitive function in the water maze test. At the age of 6 months, the vehicle of the APP/PS1 mouse model, we performed the Morris water maze test. One-way analysis of variance and post-hoc least significant difference (LSD) test were used to analyze latency in the Morris water maze test. The effect of NTR1 on the cognitive function of APP/PS1 mice at the age of 6 months was also assessed by using the Morris water maze test (Fig. 2A). The low and high dose NTR1-treated APP/PS1 mice demonstrated improved spatial memory compared to the vehicle treated APP/PS1 mice (Fig. 2A), finding the hidden platform more rapidly when the training proceeded. The NTR1 high dose treated APP/PS1 mice showed a better performance than the NTR1 low dose treated mice but failed to reach significant difference (p>0.05). In the probe test, the C57 mice searched the target quadrant intently, whereas the vehicle-treated APP/PS1 mice swam around in the tank without knowing the previous platform location (Fig. 2C, D). The NTR1 treated groups searched preferentially in the target quadrant, showing an improved memory. At the same time, the number of annulus crossings and the time spent in the target quadrant were increased significantly in the NTR1 treated APP/PS1 mice compared with the vehicle-treated APP/PS1 mice (Fig. 2B, C). The four groups of mice had no significant difference in their swimming speed or the escape latency in the visible platform test (Fig. 2E, F), indicating that visual and locomotory function did not differ among them. These results indicated that administration of NTR1 with both doses for 3 months ameliorated the spatial learning/memory of APP/PS1 mice at the age of 6 months.

3. RESULTS

3.1. NTR1 Ameliorated the Learning Deficit in the APP/PS1 Mice

To evaluate the effect of NTR1 on the cognitive function of the APP/PS1 mouse model, we performed the Morris water maze test. At the age of 6 months, the vehicle-treated APP/PS1 mice showed impaired cognitive function in the hidden-platform test with high escape latency. The low and high dose NTR1-treated APP/PS1 mice demonstrated improved spatial memory compared to the vehicle treated APP/PS1 mice (Fig. 2A), finding the hidden platform more rapidly when the training proceeded. The NTR1 high dose treated APP/PS1 mice showed a better performance than the NTR1 low dose treated mice but failed to reach significant difference (p>0.05). In the probe test, the C57 mice searched the target quadrant intently, whereas the vehicle-treated APP/PS1 mice swam around in the tank without knowing the previous platform location (Fig. 2C, D). The NTR1 treated groups searched preferentially in the target quadrant, showing an improved memory. At the same time, the number of annulus crossings and the time spent in the target quadrant were increased significantly in the NTR1 treated APP/PS1 mice compared with the vehicle-treated APP/PS1 mice (Fig. 2B, C). The four groups of mice had no significant difference in their swimming speed or the escape latency in the visible platform test (Fig. 2E, F), indicating that visual and locomotory function did not differ among them. These results indicated that administration of NTR1 with both doses for 3 months ameliorated the spatial learning/memory of APP/PS1 mice at the age of 6 months.

3.2. The Level of ChAT in APP/PS1 Mice was Restored by NTR1 Administration

ChAT is important for learning and memory because of its critical role in acetylcholine synthesis. Diminished ChAT activity occurs in AD brains and is associated with the accumulation of Aβ [4, 30-32]. To find out whether NTR1 has a protective effect on neuropathology such as ChAT deficit, we examined the ChAT level in the cortex and hippocampus of mice treated with NTR1. As we demonstrated here, the density of brown ChAT stain on the cortex of the vehicle treated APP/PS1 mice was less as compared with the C57 mice at the age of 6 months (Fig. 3A). Such decrease in the ChAT level was restored by the administration of NTR1. The ChAT level in the cortex of
the mice was also detected by western blotting (Fig. 3B, C). Consistently, the ChAT level in the vehicle treated APP/PS1 mice decreased markedly compared with the C57 mice and was restored significantly by NTR1 treatment. The high-dose treatment of NTR1 restored the ChAT of the APP/PS1 mice to the level almost same as the C57 mice. These data suggested that NTR1 may ameliorate the cholinergic function by preventing ChAT deficit in the APP/PS1 mice.

**3.3. The Effect of NTR1 on Aβ Accumulation of the APP/PS1 Mice**

The accumulation of Aβ in brain is of importance in AD pathology. To evaluate the effect of NTR1 on the Aβ accumulation, the Aβ levels in the cortex and hippocampus were analyzed by IHC and ELISA. At the age of 6 months, as revealed by IHC staining (Fig. 4A), the vehicle-treated APP/PS1 mice had developed Aβ plaques in the cerebral cortex and the hippocampus. The number and area of the plaques were significantly reduced in the NTR1 treated APP/PS1 mice in a dose-dependent manner. The number and the area of plaques were analyzed by Image J software and demonstrated in a histogram (Fig. 4B, C). Similarly, the ELISA results showed that the levels of Aβ1-40 and Aβ1-42 reduced significantly in a dose-dependent manner in both of the cortex and the hippocampus of the NTR1 treated APP/PS1 mice compared with the vehicle treated APP/PS1 mice (Fig. 5). Together, these data suggested an efficient protective effect of NTR1 on retarding Aβ accumulation in the APP/PS1 mice brains.
3.4. The Effect of NTR1 on Aβ Metabolism of the APP/PS1 Mice

To further investigate how NTR1 decreases Aβ level in the brains of the APP/PS1 mice, we investigated the NTR1’s effect on Aβ metabolism. We found that in the cortex of the APP/PS1 mice, there was no significant difference of APP expression between the NTR1 treated and the vehicle treated APP/PS1 mice (Fig. 6A, B). As shown by western blotting, the expression of IDE, a main degrading enzyme of Aβ, was decreased markedly in the vehicle treated APP/PS1 mice as compared with the C57 mice. Interestingly, the NTR1 treatment significantly elevated the expression of IDE in the APP/PS1 mice (Fig. 6A, C). The result from IHC also confirmed that the expression of IDE was increased in the cortex of the NTR1 treated APP/PS1 mice (Fig. 6D).

Furthermore, reverse transcription-PCR was performed to find out whether NTR1 has an effect on IDE transcription. Our result showed that NTR1 treated APP/PS1 mice exhibited an up-regulation of IDE mRNA as compared with the vehicle treated APP/PS1 mice (Fig. 6E, F). These data indicated that instead of affecting the APP expression level, NTR1 up-regulated the level of IDE in the APP/PS1 mice and thereby enhanced the Aβ clearance and inhibited the Aβ accumulation.

3.5. NTR1 Reduced Aβ Accumulation in N2a-APP695sw Cell Line

To further confirm the effect of NTR1 on Aβ accumulation, we treated N2a-APP695sw cells with NTR1 (0-100 µM) for 48 h. The levels of Aβ1-40 and Aβ1-42 in the cell medium were measured by ELISA. We found that both Aβ1-40 and Aβ1-42 levels were decreased by NTR1 treatment in a dose-dependent manner (Fig. 7A, B), suggesting that NTR1 had an inhibitory effect on Aβ accumulation in this Aβ overexpressing cell model.

3.6. NTR1 Increased the Level of IDE by Up-Regulating PPARγ in N2a-APP695sw Cell Line

N2a-APP695sw cells were treated with varietal doses of NTR1 for 8 h and the expression of IDE in the cells was measured by western blotting. The experiment showed that, as expected, the expression of IDE was elevated significantly by NTR1 treatment as compared with the vehicle treated group (Fig. 8A, B). It had been reported that PPARγ regulates IDE expression by binding to a peroxisome proliferator-response element in the IDE promoter. To explore the possible mechanism of NTR1 on IDE up-regulation, we examined whether the elevation of IDE induced by NTR1 was mediated by PPARγ in the N2a-APP695sw cells. Western blotting showed that the expression of PPARγ in N2a-APP695sw cells was increased by NTR1 treatment in a time- (Fig. 8D, E) and dose- (Fig. 8A, C) dependent manner. Furthermore, pre-incubation with 20 µM GW9662 significantly inhibited the NTR1 induced IDE elevation (Fig. 8F, G). These data suggest that NTR1 may enhance IDE expression, at least in part, by up-regulating the level of PPARγ.

4. DISCUSSION

IDE is a ~110 kDa zinc endopeptidase and has a variety of substrates including insulin, insulin-like growth factors I and II, glucagon, atrial natriuretic factor (ANF),
The inhibitive role for IDE in Aβ accumulation has been supported by many studies. For example, the IDE knockout mice have increased Aβ accumulation in the brain and the primary neurons derived from these mice are less effective at degrading radiolabeled Aβ [8]. Overexpression of IDE in human APP transgenic mice resulted in a significant decrease in the levels of soluble and insoluble Aβ [34]. Therefore, increasing IDE level is considered a possible therapeutic approach of AD although the changes of IDE level in AD brains remain controversial and uncertain [7, 34].

PPARγ is a key player in glucose homeostasis and insulin sensitization [35, 36] and is found to transcriptionally regulate the expression of IDE [28]. This may explain why thiazolidinediones (TZDs), a class of PPARγ agonists for the treatment of type 2 diabetes mellitus, could decrease Aβ level and Aβ induced neural toxicity and have some protective effects on AD [37, 38]. However, TZDs have several side effects such as heart failure and stroke [39]. Thus, TZDs may not be suitable for the treatment of AD patients who are elder people.

Fig. (6). Effect of NTR1 on the expression of IDE in the cortex of the mice. (A) The western blotting for APP and IDE in the cortex of the mice. Beta-actin is the loading control for APP, and GAPDH is the loading control for IDE. (B, C) Quantification of the results shown in A and depicted as -fold change over base line (C57 mice+vehicle) (n=4, *P<0.05, **P<0.01). Specially, the level of APP in the vehicle treated APP/PS1 mice was served as base line because the 6E10 antibody could not recognize the endogenous APP of the mice. (D) Immunostaining for IDE in the cortex of mice. The representative sections from each group were shown (Scale Bar=40 µm). (E) The reverse transcription-PCR for IDE. (F) Quantification of the results shown in E and depicted as fold increased (n=5-6, *P<0.05, **P<0.01).

Fig. (7). Effect of NTR1 on Aβ accumulation in N2a-APP695sw cells. (A, B) Aβ1-40(42) ELISA for conditioned medium of N2a-APP695sw cells. Cells were treated with NTR1 (0-100 µM) for 48 h and the conditioned medium was collected for ELISA (n=4, *P<0.05, **P<0.01, ***P<0.001, NS: non significance).
NTR1 is the unique and main active ingredient of *Panax notoginseng*, a traditional Chinese medicine herbal remedy which has been used for thousands of years with few side effects. Our previous study indicated that NTR1 could permeate blood-brain barrier (BBB) [40]. Another study by us suggested a protective role of NTR1 on preventing excitatory toxicity on neurons [41].

In the present study, we showed the protective effect of NTR1 on the *Aβ* over-production APP695sw/PS1ΔE9 (APP/PS1) mouse model which was reported to exhibit memory impairment at the age of 3 month and form *Aβ* plaques at the age of 5 month [42]. First, we demonstrated that 3 months administration of NTR1 improved the spatial learning and memory of the APP/PS1 mice at 6 month of age. The level of ChAT in the cortex of the APP/PS1 mice was decreased and restored upon NTR1 treatment, suggesting a protective effect of NTR1 on the AD related neuropathology.

Second, our results showed that both *Aβ*<sub>1-40</sub> and *Aβ*<sub>1-42</sub> accumulation in the cortex and the hippocampus of the APP/PS1 mice was ameliorated by NTR1 treatment. It was reported that in this APP/PS1 mice model, the amount of *Aβ* in the cortex was close to that in the hippocampus [43], which was in accordance with our results. Considering the toxic effect of *Aβ*, *Aβ* reduction after NTR1 treatment may contribute to its protective effect on learning and memory and ChAT deficit. We further showed that the level of IDE, the *Aβ* clearance enzyme, was increased by NTR1 treatment in the APP/PS1 mice. Thus, NTR1 may inhibit *Aβ* accumulation via up-regulation of IDE and exert its neuronal protective effects.

Third, we used N<sub>3</sub>a-APP695sw cell line to verify the inhibitive effect of NTR1 on *Aβ* accumulation and probed the possible mechanism. Consistently, our results showed that the levels of *Aβ*<sub>1-40</sub> and *Aβ*<sub>1-42</sub> in the conditioned medium were decreased significantly by NTR1 treatment in a dose-dependent manner. *Aβ* expression was also increased upon NTR1 treatment. Furthermore, we found that NTR1 could up-regulate the expression of PPARγ and promote the IDE transcription. Co-incubation with PPARγ antagonist GW9662 prevented NTR1 from elevating the IDE expression. These findings indicated that NTR1 may increase IDE expression via elevating the level of PPARγ and thereby boost the clearance of *Aβ* and prevent *Aβ* accumulation.

Together, our results provided evidence that NTR1 has a protective effect on learning and memory of the APP/PS1 mouse model. Moreover, NTR1 has an inhibitive effect on *Aβ* accumulation probably by up-regulating IDE level, which is mediated by PPARγ pathway. Thus, our findings suggest that NTR1 may be used as an IDE up-regulator which has a potential protective effect on AD.

**LIST OF ABBREVIATIONS**

AD = Alzheimer’s Disease

APP = Amyloid Precursor Protein
Protective Effect of Notoginsenoside R1 on an AD Mouse Model

\[ \text{Aβ} = \beta\text{-Amyloid Protein} \]
\[ \text{ChAT} = \text{Choline Acetyltransferase} \]
\[ \text{IDE} = \text{Insulin Degrading Enzyme} \]
\[ \text{IHC} = \text{Immunohistochemistry} \]
\[ \text{NTR1} = \text{Notoginsenoside R1} \]
\[ \text{PATH} = \text{Panax Notoginsenosidum} \]
\[ \text{PPARγ} = \text{ Peroxisome Proliferator-Activated Receptor γ} \]
\[ \text{TZDs} = \text{Thiazolidinediones} \]

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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