TL-2 attenuates β-amyloid induced neuronal apoptosis through the AKT/GSK-3β/β-catenin pathway

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Abstract

β-amyloid (Aβ)-mediated neuronal apoptosis contributes to the progression of Alzheimer’s disease (AD), although the exact mechanism remains unclear. This study aimed to investigate whether Dalesconol B (TL-2), a potent immunosuppressive agent with an unusual carbon skeleton, could inhibit Aβ-induced apoptosis in vitro and in vivo and to explore the underlying mechanisms. Aβ42 was injected to bilateral hippocampus of mice to make the AD models in vivo. TL-2 was able to cross the blood-brain barrier and attenuate memory deficits in the AD mice. TL-2 also inhibited Aβ42-induced neuronal apoptosis in vitro and in vivo. In addition, TL-2 could activate the AKT/GSK-3β pathway, and inhibition of AKT and activation of GSK-3β partially eliminated the neuroprotective effects of TL-2. Furthermore, TL-2 induced the nuclear translocation of β-catenin and enhanced its transcriptional activity through the AKT/GSK-3β pathway to promote neuronal survival. These results suggest that TL-2 might be a potential drug for AD treatment.

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Key words: AKT/GSK-3β/β-catenin pathway, apoptosis, β-amyloid, Dalesconol B.

Introduction

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly and is characterized by progressive deterioration in cognitive function with loss of memory. The histopathological hallmarks of AD consist of extracellular senile plaques of β-amyloid (Aβ) peptide aggregates and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein (Bettens et al., 2013). Although immunization with Aβ42 does not prevent the progressive neurodegeneration in patients with AD (Holmes et al., 2008), it is generally considered that accumulation of Aβ is the key event in AD pathology and contributes to neuronal cell death and the formation of NFTs (Karran et al., 2011; Honjo et al., 2012; Reitz, 2012). In addition, apoptosis is a critical feature in neurodegenerative disorders and Aβ is a leading candidate for activation of apoptotic mechanisms in AD (Copani et al., 1999; Dickson, 2004; Gilbert, 2013). A single administration of Aβ1–42 into the bilateral hippocampus of mice induces impairment of memory and is a useful tool for the investigation of AD (Zhao et al., 2013; Tang et al., 2014).

It has been shown that Aβ activates apoptotic cascades via extracellular Aβ directly binding to cell receptors or via accumulation of intracellular Aβ in cell organelles, such as endoplasmic reticulum or endosomes, which may trigger endoplasmic reticulum stress or mitochondrial stress (Muirhead et al., 2010; Rohn, 2010). Several signalling pathways might be involved in Aβ-induced neuronal apoptosis, including the MAPK, PI3K/AKT, NF-κB and Wnt pathways (Morishima et al., 2001; Garrido et al., 2002; Modi et al., 2012; Kavitha et al., 2013; Silva-Alvarez et al., 2013; Zhao et al., 2013). AKT induces the phosphorylation of glycogen synthase kinase 3β (GSK-3β) on serine 9, suggesting the inactivation of GSK-3β. In addition, inhibition of GSK-3β blocks the phosphorylation of β-catenin in the cytoplasm, which in turn translocates to the nucleus, binding to a member of the DNA binding protein family, the lymphoid enhancer factor-T cell factor (LEF/TCF), and induces the transcription of Wnt-response genes, such as cyclinD1 and c-myc (Kim et al., 2013).

Increased activation of the AKT/GSK-3β/β-catenin pathway provides protective effects against neuronal injury. The neurotoxicity of Aβ is associated with increased
levels of GSK-3β and loss of β-catenin in hippocampal neurons (Fuentesalba et al., 2004). Low-power laser irradiation (LPLI) attenuates Aβ-induced apoptosis partially through the AKT/GSK-3β/β-catenin pathway (Liang et al., 2012). Lithium and rosiglitazone reduce spatial memory impairment and astrocytic and microglia activation induced by amyloid burden, due to the increase of β-catenin and inhibition of GSK-3β (Toledo and Inestrosa, 2010). Huperzine A activates Wnt/β-catenin signalling and reduces amyloidosis in AD brains (Wang et al., 2011).

Dalesconol B (TL-2), a bioactive metabolite isolated from the culture of an ascomycete Daldinia eschscholzii, is a potent immunosuppressive agent with an unusual carbon skeleton (Zhang et al., 2008; Snyder et al., 2010; Fang et al., 2012). Previous studies have demonstrated the anti-inflammatory effects of TL-2 and suggested that TL-2 might alleviate the progress of neurodegenerative diseases associated with microglia activation (Han et al., 2013). In the current study, it was demonstrated that TL-2 could activate the AKT/GSK-3β/β-catenin pathway and inhibit Aβ42-induced apoptosis in vivo and in vitro, and attenuate memory impairment in AD mice.

Method

Aβ1–42 induced AD mouse model and TL-2 treatment

The Aβ1-42 (Millipore, USA) was dissolved in 1% NH4H2O at a concentration of 1 μg/μl and incubated at 37 °C for five days to allow for fibril formation. TL-2 was extracted and isolated from mantis-associated fungus Daldinia eschscholzii IFB-TL01 as previously described (Zhang et al., 2008). TL-2 was dissolved in DMSO for in vitro studies and dosed at 10% (v/v) DMSO and 90% (v/v) saline for in vivo studies. Aβ1-42 (4 μg, i.c.v) was injected into the bilateral hippocampus of the male ICR mice (weight range: 15-20 g), and then TL-2 was injected i.p. The mice were randomly assigned to four groups: the normal mice with saline or TL-2 (10 mg/kg/day, i.p. for 15 days), and Aβ1-42-induced AD mice with saline or TL-2 (10 mg/kg/day, i.p. for 15 days). All animal experiments were approved by the Animal Care Committee of Nanjing University and performed according to institutional guidelines. Every effort was made to minimize the number of mice used and their suffering.

TL-2 detection in the brain and blood of mice

Mice were anesthetized with 1% sodium pentobarbital and transcardially perfused with 0.9% saline. The brains were rapidly removed, rinsed with cold saline and homogenized, and then the supernatant was collected 6 h after TL-2 treatment (30 mg/kg, i.p.). TL-2 in all the samples was extracted by ethyl acetate and dissolved in absolute methanol for the analysis using liquid chromatography-mass spectrometry (LC-MS).

Cell culture and treatment

Primary cortical neurons were prepared as described previously (Zhu et al., 2012). Briefly, cortices of E15-17 mouse embryo were dissected and plated at 4 × 10⁶ cells/ml on poly-D-lysine-coated plates. Cells were maintained in neurobasal media supplemented with B27 (Invitrogen, USA) and 25 nm glutamine at 37 °C in a humidified 5% CO2 incubator. The purity of neurons was over 95%. The cells were treated at day eight. SH-SY5Y cells were obtained from American Type Culture Collection (ATCC) and maintained in DMEM containing 10% heat-inactivated foetal bovine serum (FBS), 2 mmol/l of L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin at 37 °C in a humidified 5% CO2 incubator. LY294002 and FH535 were purchased from Sigma (USA). The plasmids were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The human amyloid beta precursor protein 695 (APP695) overexpressing plasmid, pCB6-APP/swe, was a kind gift of Dr Jianzhi Wang (Huazhong University of Science and Technology, China). For stable transfection, pCB6-APP/swe was transfected into SH-SY5Y cells followed by G418 selection (800 μg/ml) to generate the SH-SY5Y/ APPswe cells. The GSK-3β mutant plasmid GSK-3β-S9A, which exhibited constitutively active GSK-3β, was purchased from Addgene (USA).

MTT assay

Cell viability was determined using the conventional MTT assay. After treatment, primary cortical neurons or SH-SY5Y cells were treated with 0.5 mg/ml MTT for 4 h at 37 °C. The formazan crystals were dissolved in 100 μl of DMSO and the absorbance was measured at 570 nm in a plate reader. Cell survival rates were expressed as percentages of the value of normal cells.

Apoptotic assay by flow cytometry

Apoptosis was determined by Annexin V-FITC apoptosis detection kit (KeyGen Biotech, China). After treatment, the cells were rinsed twice with PBS, centrifuged at 600 g for 10 min and resuspended in 0.5 ml binding buffer containing 5 μl Annexin V and 5 μl propidium iodide (PI), and then incubated for 15 min at 37 °C in the dark. The apoptotic rate was examined by flow cytometry.

Western blotting

Equal amounts of protein were separated by SDS-PAGE and electrophoretically transferred onto PVDF membranes. Membranes were blocked with 5% non-fat dry milk for 1 h and incubated overnight at 4 °C with rabbit anti-cleaved caspase-3 (1:1000 Cell Signalling), rabbit anti-caspase-3 (1:500, Bioworld), rabbit anti-caspase-9 (1:1000, Cell Signalling), rabbit anti-Bax (1:500, Bioworld), rabbit anti-Bcl-2 (1:500, Bioworld), mouse
anti-cytchrome c (1:500, Abcam), rabbit anti-p-AKT (1:1000, Cell Signalling), rabbit anti-AKT (1:1000, Cell Signalling), rabbit anti-p-GSK3β (1:1000, Cell Signalling), rabbit anti-GSK3β (1:1000, Cell Signalling), rabbit anti-p-β-catenin (1:1000, Cell Signalling), rabbit anti-β-catenin (1:1000, Cell Signalling), rabbit anti-p-Tau (1:500, Bioworld), rabbit anti-Tau (1:500, Bioworld), or mouse anti-GAPDH (1:500, Bioworld) antibody. GAPDH was used as a loading control. The proteins were detected with HRP-conjugated anti-rabbit or anti-mouse secondary antibodies and visualized with chemiluminescence reagents provided with the ECL kit (Bioworld). The intensity of the blots was quantified with densitometry.

Caspase-9 and -3 activity assay

The activities of caspase-3 and caspase-9 were measured by colorimetric assay kits according to the manufacturer’s instructions (KeyGen BioTech, China). In brief, SH-SY5Y cells were harvested and incubated with 50 μl lysis buffer on ice for 30 min, followed by centrifugation at 10 000 g for 1 min at 4 °C. Then, cells were suspended in 50 μl 2× reaction buffer and 5 μl caspase-3 or caspase-9 substrate incubating for 4 h at 37 °C. Later, the absorbance was read in a microplate reader at 400 nm.

Luciferase reporter activity assays

pTopFlash and phRL-CMV Renilla were cotransfected using Lipofectamine 2000 to SH-SY5Y or SH-SY5Y/APPswe cells followed by TL-2 treatment for 6 h. The luciferase activity was assayed using the Promega Bright-N-Glo system as previously described (Zhu et al., 2012). All data points were the averages of at least four independent transfections.

Confocal laser scanning microscopy

SH-SY5Y and SH-SY5Y/APPswe cells were seeded on cover slips and were fixed in 4% paraformaldehyde for 20 min at room temperature. The cells were incubated with anti-β-catenin (1:250, Cell Signalling) at 4 °C overnight and then incubated with the secondary antibody (1:500, Invitrogen) for 45 min at room temperature. Fluorescent images were taken using a confocal laser-scanning microscope (Olympus, Japan).

Morris water maze test

The Morris water maze (MWM) test was conducted as previously described (Zhu et al., 2013). Briefly, mice were trained to find a transparent Plexiglas platform in the pool placed 2 cm below the water surface in the middle of one quadrant. Four training trials per day were conducted for 5 days. In each trial, the latency to escape onto the platform was recorded up to 60 s. If a mouse could find the platform, it was allowed to remain on the platform for 5 s, and then was returned to the home cage. If a mouse was unable to find the platform within 60 s, it was gently guided to the platform and allowed to remain on the platform for 10 s, and the latency was recorded for 60 s. On the sixth day, the probe trial was conducted for memory retention by removing the platform, and each mouse was allowed to swim freely for 60 s. The number of crossings of the platform, latency to reach the platform position and time spent in each quadrant were recorded and analysed. All data were recorded with a computerized video system.

Statistical analysis

The data were expressed as mean±S.E.M and analysed by SPSS 12.0 statistical analytical software (SPSS, USA). Group differences in the escape latency during the training trial were analysed using two-way analysis of variance (ANOVA) with repeated measures followed by Bonferroni multiple comparison test with day and treatment as the sources of variation. All other data were analysed with a one-way ANOVA followed by Bonferroni’s post-hoc. Values of p<0.05 were considered statistically significant.

Results

TL-2 decreases Aβ1–42-induced neural apoptosis

Preliminary data indicated that TL-2 had no effect on the viability of SH-SY5Y cells, whereas the cell viability was significantly decreased under Aβ1–42 treatment (p<0.05, Fig. S1a, b). In addition, TL-2 could significantly increase the cell viability of SH-SY5Y cells and primary cortical neurons (p<0.05, Fig. S1c, d).

To explore whether TL-2 could protect SH-SY5Y cells against Aβ1–42-induced apoptosis, Annexin V/PI staining was performed using flow cytometric analysis. As shown in Fig. 1a, b, Aβ1–42 treatment significantly increased the cell apoptotic rate in Aβ1–42-incubated SH-SY5Y cells, while TL-2 could significantly attenuate the apoptotic rate (p<0.01). In addition, TL-2 could decrease the activities of caspase-9 and caspase-3 in Aβ1–42 -incubated SH-SY5Y cells (p<0.01, Fig. 1c, d).

Activation of AKT/GSK3β partially contributes to the anti-apoptosis role of TL-2

Since emerging evidence suggested that the AKT/GSK3β pathway played a critical role in Aβ-induced apoptosis, the current study investigated whether TL-2 exerted its anti-apoptosis effect by modulating the AKT/GSK3β pathway. As shown in Fig. 2a, Aβ1–42 treatment decreased the phosphorylation of AKT on serine 473 and increased the phosphorylation of GSK3β on serine 9, which indicated the inactivation of the AKT/GSK3β pathway. However, TL-2 treatment could up-regulate the AKT/GSK3β pathway in Aβ1–42-incubated SH-SY5Y cells. To explore whether activation of the AKT/GSK3β pathway contributed to the anti-apoptosis effects of TL-2, a specific inhibitor of AKT, LY294002, was used. As shown in Fig. 2b, LY294002 inhibited the phosphorylation of AKT and to
GSK-3 vs. ##: p

μ20 (Fig. 2. Activation of AKT/GSK3

TL-2 decreases Aβ1-42-induced neural apoptosis. (a) SH-SY5Y cells were pre-treated with 50 μM TL-2 and then incubated with 20 μM Aβ1-42 for 6 h, and the apoptotic rate was determined by AnnexinV/PI staining. (b) Quantitative analysis of apoptotic cells. The activities of caspase-9 (c) and caspase-3 (d) were measured in TL-2 and Aβ1-42 treated SH-SY5Y cells by means of colorimetric assay kits. **: p<0.01 vs. con; ##: p<0.01 vs. Aβ.

Fig. 2. Activation of AKT/GSK3β partially contributes to the anti-apoptotic role of TL-2. (a) SH-SY5Y cells were pre-treated with 40, 60 and 80 μM of TL-2 and then incubated with 20 μM Aβ1-42 for 6 h, and the activity of AKT/GSK3β pathway were determined by Western blotting. **: p<0.01 vs. con; #: p<0.01 vs. Aβ. (b) The activity of AKT/GSK3β pathway in SH-SY5Y cells pre-treated with LY294002 (10 μM) was determined by Western blotting. **: p<0.01 vs. con; #: p<0.01 vs. Aβ; &: p<0.01 vs. Aβ+TL-2. (c) The apoptotic rate in LY294002 (10 μM) pre-treated SH-SY5Y cells was determined by AnnexinV/PI staining. **: p<0.01 vs. control; #: p<0.01 vs. Aβ; &: p<0.01 vs. Aβ+TL-2. (d) The activity of GSK3β in SH-SY5Y cells transfected with GSK-3β-S9A was determined by Western blotting. **: p<0.01 vs. con; #: p<0.01 vs. Aβ; &: p<0.01 vs. Aβ+TL-2. (e) The apoptotic rate in GSK-3β-S9A transfected SH-SY5Y cells was determined by AnnexinV/PI staining. **: p<0.01 vs. control; #: p<0.01 vs. Aβ; &: p<0.05 vs. Aβ+TL-2.
increased the phosphorylation of GSK3β, which indicated the inactivation of the AKT/GSK3β pathway. LY294002 treatment also significantly increased the apoptotic rate in TL-2 and Aβ₁₋₄₂-treated SH-SY5Y cells (Fig. 2c). In addition, GSK-3β-S9A, a constitutively active form of GSK-3β, was transfected into SH-SY5Y cells followed by TL-2 and Aβ₁₋₄₂ treatment. As shown in Fig. 2d, e, overexpression of GSK-3β inhibited the phosphorylation of GSK3β, while it could also increase the apoptotic rate in TL-2 and Aβ₁₋₄₂-treated SH-SY5Y cells. These results collectively demonstrated that activation of the AKT/GSK3β pathway contributed to the anti-apoptosis role of TL-2 in Aβ₁₋₄₂-treated SH-SY5Y cells.

**Activation of β-catenin participates in the anti-apoptosis role of TL-2**

Previous studies have demonstrated that loss of β-catenin function is involved in Aβ-dependent neurodegeneration in the pathogenesis of AD. As shown in Fig. 3a, TL-2 could induce the transcriptional activity of β-catenin in SH-SY5Y and SH-SY5Y/APPswe cells. The results of Western blotting also suggested that Aβ₁₋₄₂ treatment increased the phosphorylation of β-catenin and decreased the expression of β-catenin, and TL-2 could partially reverse these effects (Fig. 3b). Furthermore, TL-2 treatment markedly induced the accumulation of β-catenin in the nuclei of SH-SY5Y/APPswe cells (Fig. 3c). These results indicated that TL-2 could increase the expression of β-catenin and induce the nuclear translocation of β-catenin in SH-SY5Y/APPswe cells. To verify whether activation of β-catenin contributed to the anti-apoptosis role of TL-2, FH535, a reversed inhibitor of β-catenin, was used. As shown in Fig. 3d, FH535 treatment significantly attenuated the anti-apoptosis effects of TL-2. These results suggested that activation of β-catenin was involved in the anti-apoptosis role of TL-2 in SH-SY5Y/APPswe cells.

**TL-2 decreases the apoptotic rate and activates the AKT/GSK3β/β-catenin pathways in vivo**

Since TL-2 was able to cross the blood-brain barrier (Fig. S2), we also examined the effect of TL-2 on Aβ₁₋₄₂-induced apoptosis in vivo. As shown in Fig. 4a, TL-2
Treatment increased the expression of Bcl-2 and decreased the expression of Bax. It also markedly inhibited the release of cytochrome c in Aβ1–42-induced AD mice. In addition, TL-2 could inhibit the activation of caspase-9 and caspase-3 in AD mice. Furthermore, the effect of TL-2 on the AKT/GSK3β/β-catenin pathway was also examined in the AD mice. As shown in Fig. 4b, Aβ1–42 decreased the phosphorylation of AKT and GSK3β, and increased the phosphorylation of β-catenin, which indicated that Aβ1–42 inactivated the AKT/GSK3β/β-catenin pathway. However, TL-2 treatment could activate the AKT/GSK3β/β-catenin pathway in AD mice.

**TL-2 attenuates the memory impairment in Aβ1–42-induced AD mice**

It was reported that TL-2 exerted a potent immunosuppressive effect comparable to that of CsA (Zhang et al., 2008). Results of the current study indicated that short-term treatment of TL-2 did not affect the body weights, weights of spleen, the numbers of WBC, hemoglobin, blood platelet and erythrocyte in Aβ1–42 induced AD mice (Fig. S3). The effect of TL-2 (10 mg/kg, i.p. for 15 d) on learning and memory was investigated using the MWM test. As shown in Fig. 5a, the mean escape latency of Aβ1–42-induced AD mice was significantly increased compared with control ICR mice (p<0.01), while TL-2-treated AD mice showed significant improvements compared with AD mice after the training periods (p<0.01). On the sixth day, the platform was removed and the probe trial was conducted. TL-2 significantly decreased the escape latency of AD mice and the number of platform crossings by the TL-2-treated AD mice was significantly higher than that of AD mice (p<0.05, Fig. 5b, c). Moreover, TL-2-treated AD mice spent more time in the target quadrant than AD mice (p<0.05, Fig. 5d). Thus,
these results demonstrated that TL-2 treatment significantly improved the memory deficits in AD mice.

**Discussion**

**Aβ**, the major component of senile plaques, plays a critical role in the development of AD. The molecular mechanisms underlying Aβ-mediated neuronal apoptosis remain unclear. This study, for the first time, has demonstrated that TL-2, a kind of polyketide derived from extract of scaled-up fermentation of *Daldinia eschscholzii*, has neuroprotective effects against Aβ-induced neuronal apoptosis and attenuates memory deficits in Aβ-induced AD mice, which might result from activation of the AKT/GSK-3β pathway.

Increasing evidence has indicated that Aβ is able to induce neuronal apoptosis. Under Aβ stimulation, the mitochondrial permeability transition pore (PTP) tends to open and the mitochondrial membrane potential is depolarized. The balance between anti-apoptotic protein (Bcl-2, Bcl-xl) and pro-apoptotic protein (Bax, Bad, Mcl-2) is disturbed, which triggers cytochrome c release. Subsequent release of cytochrome c leads to oligomerization of apoptotic protease activating factor-1 (Apaf-1) and promotes the activation of caspase9 and caspase3, which results in both DNA fragmentation and membrane phosphatidylserine exposure (Rohn, 2010; Eckert et al., 2012). In this study, it was demonstrated that TL-2 significantly decreased the apoptotic rate and activities of caspase9 and caspase3 in Aβ treated SH-SY5Y cells. The results of in vivo experiments also indicated that TL-2 could decrease the level of Bax, cytosol cytochrome c, cleaved caspase9 and cleaved caspase3 in Aβ-induced AD mice. These results suggested that TL-2 could reduce Aβ-induced neuronal apoptosis in vitro and in vivo.

The AKT/GSK-3β pathway mediates various processes such as metabolic control, embryogenesis, cell death, and oncogenesis (McCubrey et al., 2013; Osolodkin et al., 2013; Wang et al., 2013). Increasing evidence shows that GSK-3β is implicated in the pathogenesis of AD. GSK-3β promotes hyperphosphorylation of the tau protein and causes the formation of NFTs, which are one of the classic hallmarks of AD (Hernandez et al., 2013). Coenzyme Q10 could rescue Aβ-inhibited proliferation of neural stem cells by activating the PI3K/AKT/GSK-3β pathway (Choi et al., 2013). Curcumin could activate the AKT/GSK-3β pathway in the presence of Aβ in organotypic hippocampal slice culture (Zhang et al., 2011). GLP-1 (9-36) (amide) restored the dysregulated AKT/GSK-3β pathway in the hippocampus of APP/PS1 mice and rescued synaptic plasticity and memory deficits (Ma et al., 2012). Propranolol could up-regulate the AKT/GSK-3β pathway and restore cognitive deficits and improve amyloid and tau pathologies in AD mice (Dobarro et al., 2013). This study validated that TL-2 could activate the AKT/GSK-3β pathway, and inhibition of AKT and activation of GSK-3β could partially eliminate the neuroprotective effects of TL-2, indicating that TL-2 exerted the anti-apoptosis effects through the AKT/GSK-3β pathway. Furthermore, GSK-3β could induce the
hyperphosphorylation of Tau at several Ser or Thr phosphorylation sites and our results demonstrated that TL-2 could decrease the levels p-Tau (Ser396) in the hippocampus of AD mice (Fig. S4), which might also contribute to the neuroprotective effects of TL-2.

$\beta$-catenin is a critical component in the downstream of the Wnt signalling pathway and acts as an intracellular signal transducer to mediate gene transcription, which can promote cell survival (Kim et al., 2013; Stamos and Weis, 2013). Reduced Wnt/$\beta$-catenin pathway has been reported in the pathophysiology of neuronal degeneration in AD (Boonen et al., 2009; Rosso and Inestrosa, 2013). In this study, it was shown for the first time that, as a result of inhibition of GSK-3$\beta$ by TL-2, $\beta$-catenin accumulated in the cytoplasm and then translocated into the nucleus where it acted as a transcriptional cofactor with TCF/LEF to promote cell survival. In addition, FH535 could partially abolish the neuroprotective effect of TL-2, which suggested that the Wnt/$\beta$-catenin pathway contributed to the neuroprotective effects of TL-2. These studies suggest the AKT/GSK-3$\beta$/$\beta$-catenin pathway as an appealing therapeutic target in treating AD, and TL-2 may have a positive impact on AD treatment via activation of the AKT/GSK-3$\beta$/$\beta$-catenin pathway.

In conclusion, the current study demonstrates that TL-2 inhibits Ab$\beta$-induced apoptosis in vitro and in vivo and attenuates the memory deficits in AD mice. Furthermore, TL-2 treatment activates the AKT/GSK-3$\beta$/$\beta$-catenin pathway, which plays an important role in the anti-apoptotic effects of TL-2. Thus, these results suggest that TL-2 might be a potential drug for the treatment of AD by activation of the AKT/GSK-3$\beta$/$\beta$-catenin pathway.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000315.

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Conflicts of Interest

None.

Reference


