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BIOMEDICAL SCIENCES

Neuroprotection of Triptolide against Amyloid-Beta$_{1-42}$-induced toxicity via the Akt/mTOR/p70S6K-mediated Autophagy Pathway

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Abstract: Triptolide is a natural active compound that has significant neuroprotective properties and shows promising effects in the treatment of Alzheimer's disease (AD). Recent studies have shown that autophagy occurs in AD. In this study, we determined whether autophagy regulated by triptolide ameliorates neuronal death caused by amyloid-Beta$_{1-42}$ (Aβ$_{1-42}$). We examined the effects of triptolide on cell viability, autophagy, apoptosis, and the protein kinase B/mammalian target of the rapamysin/70 kDa ribosomal protein S6 kinase (Akt/mTOR/p70S6K) signaling pathway in PC12 cells. The results indicated that triptolide treatment exhibited a cytoprotective effect against cell injury induced by Aβ$_{1-42}$. Triptolide also reduced apoptosis and enhanced cell survival by decreasing autophagosome accumulation and inducing autophagic degradation. Furthermore, our results also showed that activating the Akt/mTOR/p70S6K mechanism was one reason for the protection of triptolide. Triptolide treatment protected against Aβ$_{1-42}$-induced cytotoxicity by decreasing autophagosome accumulation, and inducing autophagic degradation in PC12 cells. These findings also suggest that the reduction of autophagosome accumulation observed in triptolide-treated cells was Akt/mTOR/p70S6K pathway dependent. Overall, triptolide exhibits a neuron protective effect and this study provides new insight into AD prevention and treatment.

Key words: Alzheimer's disease, triptolide, PC12 cells, apoptosis, cytotoxicity.

INTRODUCTION

Alzheimer's disease (AD) is the primary cause of dementia and is one of the most challenging diseases facing modern medicine (Lane et al. 2018). Clinical symptoms include deterioration of memory and cognitive dysfunction that interferes with daily life (Scheltens et al. 2016, Soria Lopez et al. 2019). However, the underlying cause of AD is poorly understood and there are no available treatments that can effectively manage or cure this disease.

Recent studies have demonstrated that the extracellular presence of amyloid-beta (Aβ) peptides comprising senile plaques, along with the abnormal metabolism of Aβ is characteristic of AD (Fabiani & Antollini 2019). Genetic studies, postmortem pathological examinations, and experimental studies of human and animal brains afflicted with AD have revealed that Aβ in the brain of AD patients was 1-42-amino acid Aβ (Selkoe 2001). Compared with Aβ$_{1-40}$ and Aβ$_{25-35,}$ Aβ$_{1-42}$ was the most biologically active amyloid peptide and demonstrated an enhanced propensity to form fibrils (Baldassarre et al. 2017, Itoh et al. 2018, Zhang et al. 2020). Therefore, inhibiting the production of Aβ$_{1-42}$ and reversing its cytotoxicity is one strategy for treating AD. Aducanumab, a new U.S. Food and Drug Administration (FDA) approved drug for the

treatment of AD exerts its therapeutic activity through anti-Aβ (Knopman et al. 2021).

Although age, inflammation, and oxidative stress all contribute to the onset of AD (Sinyor et al. 2020), autophagy also has a central role. Autophagy is a protein degradation system in which cellular proteins and organelles are sequestered, delivered to lysosomes, and digested by lysosomal hydrolases. Poorly functioning autophagy is associated with the metabolism and generation of Aβ, as well as the assembly of tau in AD (Li et al. 2017a). Previous studies have indicated that many autophagosomes appear in the AD brain (Pickford et al. 2008), whereas over-activated autophagy promotes neuronal damage and ultimately leads to AD. Meanwhile, galamantine, as a first-line pharmacological AD treatment inhibits Aβ-induced cytostatic autophagy by decreasing ROS accumulation as a new mechanism (Li et al. 2019, Jiang et al. 2018). Therefore, the regulation of autophagy and the resulting turnover of toxic misfolded proteins may represent a neuroprotective strategy in AD treatment, thus, autophagy modulators that maintain the autophagic flux may have therapeutic implications.

Triptolide (TP) is the primary active compound in the Traditional Chinese Medicine (TCM) herb *Tripterygium wilfordii* Hook F. (TWHF), which possesses a diverse range of immunosuppressive and anti-inflammatory properties (Chen et al. 2018). Multiple studies have shown that triptolide is an autophagy modulator and its activity is associated with the up- or down-regulation autophagy (Chan et al. 2017, Li et al. 2017b, Miao et al. 2020, Xie et al. 2019). Because of its neuroprotective effects, the therapeutic potential of triptolide for AD has attracted significant attention (Li & Hao 2019). Studies have shown that triptolide exhibits a neuroprotective effect in different animal and cellular AD models; however existing studies have focused on the impact of administered triptolide on AD-related behavior and neuropathology. For example, some studies indicate that triptolide treatment reduces Aβ deposition, attenuates neuroinflammation, enhances spatial learning performance, and prevents cognitive deficits in a mouse model of AD (Cheng et al. 2014, Wang et al. 2014). Little is known about whether triptolide has a protective effect on the cytotoxicity of differentiated PC12 cells induced by $A\beta_{1-42}$ and whether cell autophagy is involved in these processes. Our previous studies found that the neuroprotective effects of triptolide against $A\beta_{25-35}$ occurred through autophagy inhibition, which could be blocked by rapamycin (an autophagy inducer) (Xu et al. 2015). Therefore, we hypothesize that autophagy is associated with the neuroprotective effects of triptolide against neurotoxic of $A\beta_{1-42}$, which is closest to the normal pathological state of AD. In this study, we used PC12 cells that were treated with $A\beta_{1-42}$ as an experimental model to investigate the neurotoxicity of Aβ (Gao et al. 2020, Liu et al. 2018, Yang et al. 2019) and to determine whether triptolide regulates autophagy induced by Aβ. A better understanding of these processes will lead to the development of new therapeutics and pave the way for the prevention and treatment of AD.

MATERIALS AND METHODS

Ethics statement

All participants of this study have given their written informed consent. The study protocol has been approved by the committee on research at Tianjin University of Traditional Chinese Medicine (No.TCM-LAEC2020026).

Materials

Triptolide, $A\beta_{1-42}$, NGF-β and rapamycin were all obtained from Sigma Chemical Co., MO, USA.

The RPMI 1640 medium and fetal bovine serum (FBS) were obtained from Gibco BRL, USA. Rabbit monolyclonal anti-Postsynaptic density 95 (anti-PSD 95), rabbit monolyclonal anti-synaptophysin (anti-SYP), rabbit anti-Neprilysin antibody (anti-NEP), rabbit monolyclonal anti-BACE1, rabbit monoclonal anti-Beclin-1, rabbit polyclonal to Akt, rabbit monoclonal anti-S6K1, and rabbit monoclonal anti-mTOR, were purchased from Abcam, Cambridge, MA (U.S.A). The rabbit polyclonal anti-LC 3B antibody was obtained from GeneTex, Inc., CA (U.S.A). The rabbit monoclonal anti-p62/SQSTM1 antibody was obtained from Abways Technology, Inc., Shanghai, China. The rabbit anti-phosphorylated-p70S6K, rabbit monolyclonal anti-phosphorylated-Akt, and rabbit monolyclonal anti-phosphorylated-mTOR antibodies were obtained from Cell Signaling Technology, Inc. MA (U.S.A). The potent isozyme selective Akt1/2 kinase inhibitor (Akt-I-1/2) was obtained from Abcam, Cambridge, MA (U.S.A). mTOR Trilencer-27 Rat siRNA and mouse monoclonal anti-GAPDH antibody were obtained from OriGene Technologies, Inc., U.S.A. The Annexin V-FITC propidium iodide (PI) apoptosis detection kit was obtained from Beyotime Biotechnology, USA. We bought the chemiluminescent HRP substrate (Immobilon western) from Millipore Corporation, Billerica, MA, U.S.A, and the Cell Counting Kit-8 (CCK-8) assay kit was obtained from Dojindo Molecular Technologies, Inc. Shanghai, China.

Cell culture and treatment

The rat pheochromocytoma cell line (PC12, derived from a rat adrenal medulla pheochromocytoma) was purchased from the Institute of Basic Medical Sciences at the Chinese Academy of Medical Sciences, which is outlined in our prior research (Xu et al. 2015, 2016). Undifferentiated PC12 cells were cultured with 10% FBS, 1% antibiotics, and exposed to air with humidity of 95% (5% CO_2) at 37°C. We then added serum-free RPMI1640 to the gel, and as well as 50 ng/ml of nerve growth factor (NGF) for 7 days. From this, we got PC12 cells differentiated by their neurons, which were widely used in the *in vitro* models of neurons (Abubaker et al. 2019). We then incubated the cells in a RPMI 1640 medium augmented with antibiotics (1%) and FBS (5%). Each subsequent experiment was performed with the differentiated PC12 cells described here. Several researches on AD used a PC12 cell line with Aβ treatment to investigate the neurotoxicity of Aβ (Cheng et al. 2018, Hadipour et al. 2020, Jesko et al. 2018).

An $A\beta_{1-42}$ peptide stock solution (1mmol/L) in phosphate buffered saline (PBS), was prepared and kept at -20°C. It was then incubated at 37° C to aggregate before use. When used, the $A\beta_{1-42}$ was altered with varying amounts of the RPMI 1640 medium. Triptolide was dissolved in dimethylsulfoxide (DMSO) with a concentration of 2mg/ml. After reconstitution, triptolide was diluted to $10^{-10,}$ 10^{-9}, 10^{-8} mol/L with RPMI 1640 medium.

Cytotoxicity of $A\beta_{1-42}$ on PC12 cells

The toxicity caused by $A\beta_{1-42}$ was measured by observing the cell viability with the CCK-8 kit. The PC12 cells were cultured with RPMI 1640 for 24 h on 96-well plates, and treated with different levels of $A\beta_{1-42}$ (1, 5, 10 μmol/L) for 12, 24, and 36 h. Then 10 μl of the CCK-8 mix was added to each portion of the culture plate. After one hour of incubation, a Multiskan Spectrum micro-plate reader was used to establish the absorbance. For a control, the cells exposed to the culture medium was used. The viability of the cell was expressed as a viability ratio of the control. Each variable and experiment were conducted independently for at least three times.

Safety detection of triptolide

The safety detection of triptolide was analyzed using a CCK-8 kit. PC12 cells were plated in 96-well cell culture plates, and triptolide was subsequently added in the culture media at concentrations of 10^{-10}, 10^{-9}, 10^{-8} mol/L for 24 h. The viability of the cell was established using a CCK-8 kit. Each variable and experiment were conducted independently for at least three times.

Guarding effects of triptolide on cytotoxicity induced by $A\beta_{1-42}$ in various PC12 cells

We exposed differentiated PC12 cells to 10 μmol/L $A\beta_{1-42}$ in order to establish the cytotoxicity of Aβ. The cells were exposed to varying levels of triptolide for 24 h (10^{-10}, 10^{-9}, 10^{-8} mol/L) with 10 μmol/L $A\beta_{1-42}$, and a CCK-8 assay was used to establish viability. Each variable and experiment were conducted independently for at least three times.

Quantitative measurement of apoptosis with flow cytometry

Quantitative measurement of apoptosis was performed using the Annexin V-FITC and PI staining was assessed using flow cytometry. PC12 cells were seeded into the parallel wells of 6-well plates and cultured for 24–48 h. Then 10 μmol/L $A\beta_{1-42,}$ 10 μmol/L $A\beta_{1-42}$ +10^{-9} mol/L triptolide were added in the culture media for 24 h. After treatment, the cells were harvested and PI staining and Annexin V-FITC via flow cytometry (CANTO II, BD) were used to measure their apoptosis. The apoptosis ratio was calculated by adding the ratio of late apoptotic cells with the percentage of early apoptotic cells. This cell percentage is shown in the area of the respective quadrants. Each experiment was conducted at least three times.

Western blot assay

We extracted samples of protein from differentiated PC12 cells, and they were then incubated in a cell culture mix and quantified using a BCA protein assay kit according to the following specifications: 10 μmol/L $A\beta_{1-42,}$ 10 μmol/L $A\beta_{1-42}$ and 10^{-9} mol/L triptolide for 24 h. SDS-PAGE protein extracts were moved to PVDF membranes, which were exposed to primary antibodies (anti-PSD 95, anti-SYP, anti-NEP, anti-BACE1, anti-Beclin-1, anti-LC3, anti-p62/SQSTM1, anti-S6K1, anti- Akt, anti-mTOR, anti-p-p70S6K, anti-p-Akt, anti-p-mTOR) at 4°C until the next day. The membranes were washed and exposed to HRP-conjugated secondary antibodies (goat anti-rabbit HRP antibody (working dilution 1:20000) and (goat anti-mouse HRP antibody (working dilution 1:10000)) for 1 h at room temperature. An enhanced chemiluminescence assay kit (Pierce ECL Western Blotting Substrate) was used to see the bands of protein, and the equal loading of the proteins was corroborated using anti-GAPDH. Image J software was used to analyze the density of the bands. Each experiment was conducted at least three times.

Immunofluorescence

The autophagosome formation was analyzed by immunofluorescence staining of LC3. Differentiated PC12 cells were seeded in 6-well plates and treated with cell culture medium, 10 μmol/L $A\beta_{1-42}$ and /or 10^{-9} mol/L triptolide for 24 h. Next, the cells were fixed and blocked with serum, then incubated with LC3 B at 4°C overnight. After DAPI staining, the cells were immediately visualized by a Leica TCS SP5 laser-scanning confocal microscope for the detection of autophagosome formation.

Effect of inhibitor of the Akt/mTOR/p70S6K pathway

To assess the Akt/mTOR/p70S6K pathway effects of the protective properties of triptolide in $A\beta_{1-42}$ treated-PC12 cells, Akt-I-1/2 (an Akt1/2 kinase inhibitor), rapamycin (an mTOR inhibitor, 10ng/ml) and siRNA-mTOR were used to detect apoptosis and the viability of the cell.

A medium of cell culture (10 μmol/L $A\beta_{1-42}$ $+10^{-9}$ mol/L triptolide, 10 μmol/L $A\beta_{1-42}$, 10 μmol/L $A\beta_{1-42}$ $+10^{-9}$ mol/L triptolide+ 15 μmol/L Akt-I-1/2, 10 μmol/L $A\beta_{1-42}$ $+10^{-9}$ mol/L triptolide+ 10ng/ml rapamycin, 10 μmol/L $A\beta_{1-42}$ $+10^{-9}$ mol/L triptolide+ siRNA-mTOR) was incubated for 24 h. The mTOR Trilencer-27 Rat siRNA was used to perform mTOR-siRNA, according to the manufacturer's instructions.

The cell viability was determined by a CCK-8 assay, followed by a quantitative measurement of apoptosis PI and Annexin V-FITC staining were assessed with flow cytometry. Each variable and experiment were conducted three times.

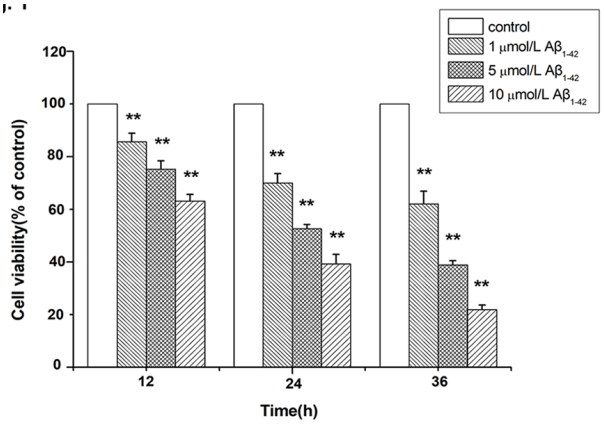

Figure 1. Cytotoxicity of $A\beta_{1-42}$ on PC12 cells. A culture media (1 μmol/L $A\beta_{1-42}$, 5 μmol/L $A\beta_{1-42}$ and 10 μmol/L $A\beta_{1-42}$) was used to treat the PC12 cells for 12, 24 and 36 h. A CCK-8 assay was used to measure the viability of the cells, while the outcomes were represented by the absorbance percentage as compared to the control group. Data represent the mean ± S.E.M. n=6/group. **P < 0.01 vs. control group.

Statistical analysis

We analyzed our results using Origin 9.0 (Origin Lab Co.) and SPSS 22.0 (IBM Co.), which were then represented as the mean ± S.E.M. Data were compared and analyzed by one-way ANOVA. P < 0.05 was considered statistically significant.

RESULTS

$A\beta_{1-42}$ lowered the viability of PC12 cells

Incubation with $A\beta_{1-42}$ significantly lowered (P < 0.01) the viability of the PC12 cell depending on both time and dose. This showed that toxicity was induced by $A\beta_{1-42}$ in PC12 cells (Fig. 1). We chose the concentration of 10 μmol/L $A\beta_{1-42}$ grown for 24 h for this experiment, according to the references (Abubaker et al. 2019) and previous experimental results.

Triptolide was safe to treat PC12 cells

When the PC12 cells were only incubated with triptolide, we did not observe a significant decrease in PC12 cell viability compared to the control group (P > 0.05) (Fig. 2). Our findings demonstrate that the concentrations of

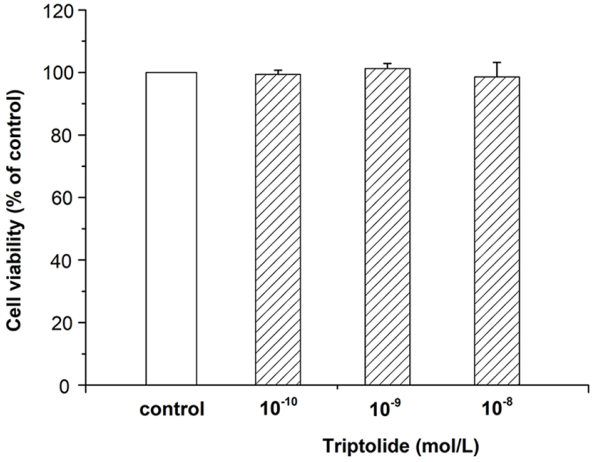

Figure 2. Safety detection of triptolide. Different concentrations of triptolide (10^{-10}, 10^{-9}, 10^{-8} mol/L) was used to treat the PC12 cells for 24 h. A CCK-8 assay was used to measure the viability of the cells. Data represent the mean ± S.E.M. n=6/group.

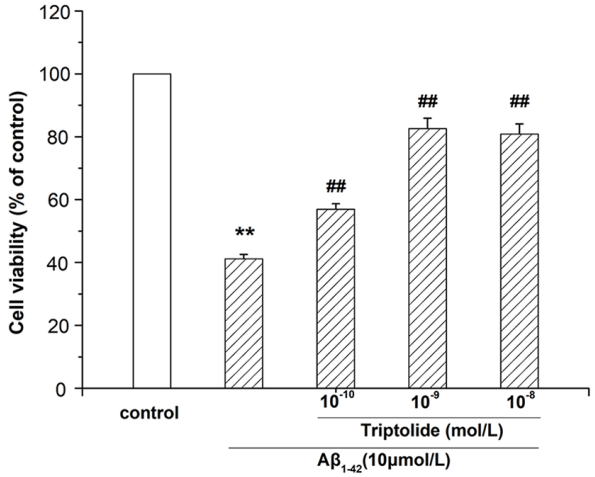

Figure 3. Protective effect of triptolide on cytotoxicity induced by $A\beta_{1-42}$. A culture media (10 μmol/L $A\beta_{1-42}$+10^{-8} mol/L triptolide, 10 μmol/L $A\beta_{1-42}$, 10 μmol/L $A\beta_{1-42}$+10^{-10} mol/L triptolide, and 10 μmol/L $A\beta_{1-42}$+10^{-9} mol/L triptolide) was used to treat the PC12 cells for 24 h. A CCK-8 assay was used to measure the viability of the cells, while the outcomes were represented by the absorbance percentage as compared to the control group. Data was calculated as the mean ± S.E.M. n=6/group. **$P < 0.01$ vs. control group. ##$P < 0.01$ vs. 10 μmol/L $A\beta_{1-42}$ group.

triptolide tested were showed no toxicity to PC12 cells.

Triptolide treatment inhibited $A\beta_{1-42}$-induced cytotoxicity in PC12 cells

The cell viability in the $A\beta_{1-42}$ group decreased significantly compared to the control group ($P < 0.01$), while triptolide exposure significantly increased the viability of the cell when compared to the $A\beta_{1-42}$ group and had a dose-dependent ($P < 0.01$, Fig. 3). These findings indicate triptolide could alleviate cellular damage caused by $A\beta_{1-42}$, suggesting that triptolide possesses neuroprotective properties.

Triptolide treatment decreased the $A\beta_{1-42}$-induced apoptosis in PC12 cells

As Fig. 4 shows, the apoptotic rate in the control group was improved significantly after treatment with $A\beta_{1-42}$ ($P < 0.01$). And triptolide markedly reduced the apoptosis ratio from 40.23% to 11.56% compared with $A\beta_{1-42}$ group ($P < 0.01$). This data was indicated that treatment with triptolide showed the protective effects from $A\beta_{1-42}$-induced cell apoptosis.

Triptolide treatment improved the synaptic function in PC12 cells

As Fig. 5 shows, our results demonstrate that the expression of PSD 95 and SYP decreased after they were incubated with $A\beta_{1-42}$ for 24 h ($P < 0.05$), indicating that triptolide could stop the reduction of PSD 95 and SYP induced by $A\beta_{1-42}$ ($P < 0.05$).

Triptolide treatment inhibited metabolism of Aβ in PC12 cells

As Fig. 6 shows, $A\beta_{1-42}$ reduced levels of NEP and increased the expression of BACE1 compared with that of control group ($P < 0.05$). Co-treatment with triptolide could significantly inhibit $A\beta_{1-42}$-induced BACE1 production ($P < 0.05$). Our findings indicate that triptolide suppresses the Aβ metabolism of PC12 cells stimulated by $A\beta_{1-42}$.

Triptolide treatment regulated $A\beta_{1-42}$-induced autophagy in PC12 cells

To evaluate the role of autophagy in the neuroprotective process of triptolide, we detect the fluorescent puncta of LC3, which indicates autophagosome formation, the expression of Beclin-1, LC3II and p62/SQSTM1. As a result, treatment with $A\beta_{1-42}$ demonstrates larger numbers of bright fluorescent particles in cells, indicating the presence of autophagosomes, while triptolide inhibits the increase in the autophagy process induced by $A\beta_{1-42}$. (Fig. 7a). We also found that $A\beta_{1-42}$ increased the expression levels of the autophagy hallmark proteins Beclin-1 and LC3II compared to those of control

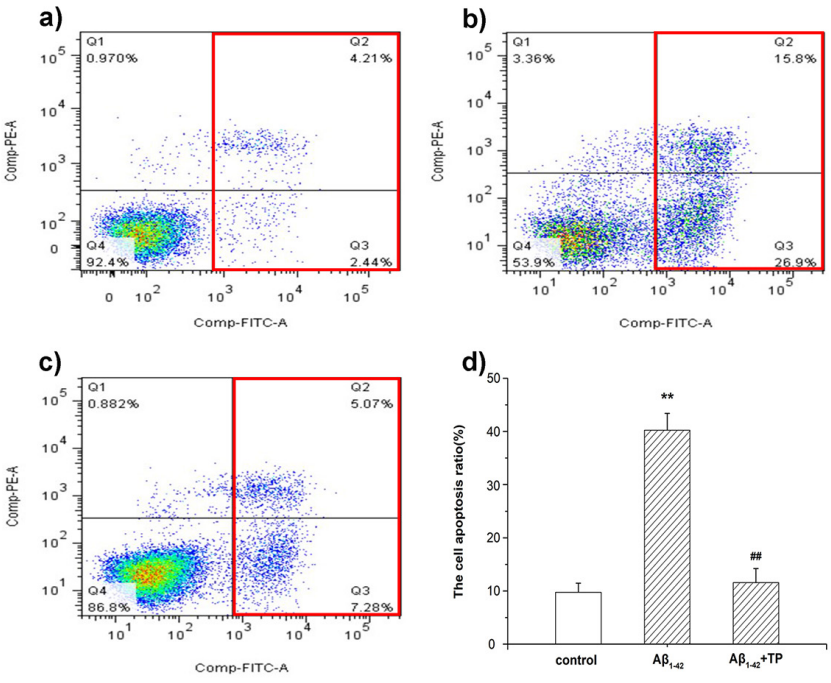

Figure 4. Protective effect of triptolide on apoptosis induced by Aβ$_{1-42}$ in PC12 cells. Flow cytometry was used to measure the apoptotic rate of PC12 cells. A culture media (culture media **(a)**, 10 μmol/L Aβ$_{1-42}$ **(b)**, and 10 μmol/L Aβ$_{1-42}$+10^{-9} mol/L triptolide **(c)**) was used to treat the PC12 cells for 24 h. The intensity of Annexin V-FITC fluorescence was on the X-axis and PI fluorescence was on the Y-axis. The bar graph was shown in **(d)**. The ratio of late apoptotic cells added with the percentage of early apoptotic cells was the apoptosis ratio. This cell percentage is shown in the area of the respective quadrants. Data was calculated as the mean ± S.E.M. Each experiment was conducted at least three times. $^{**}P < 0.01$ vs. control group. $^{\#\#}P < 0.01$ vs. 10 μmol/L Aβ$_{1-42}$ group.

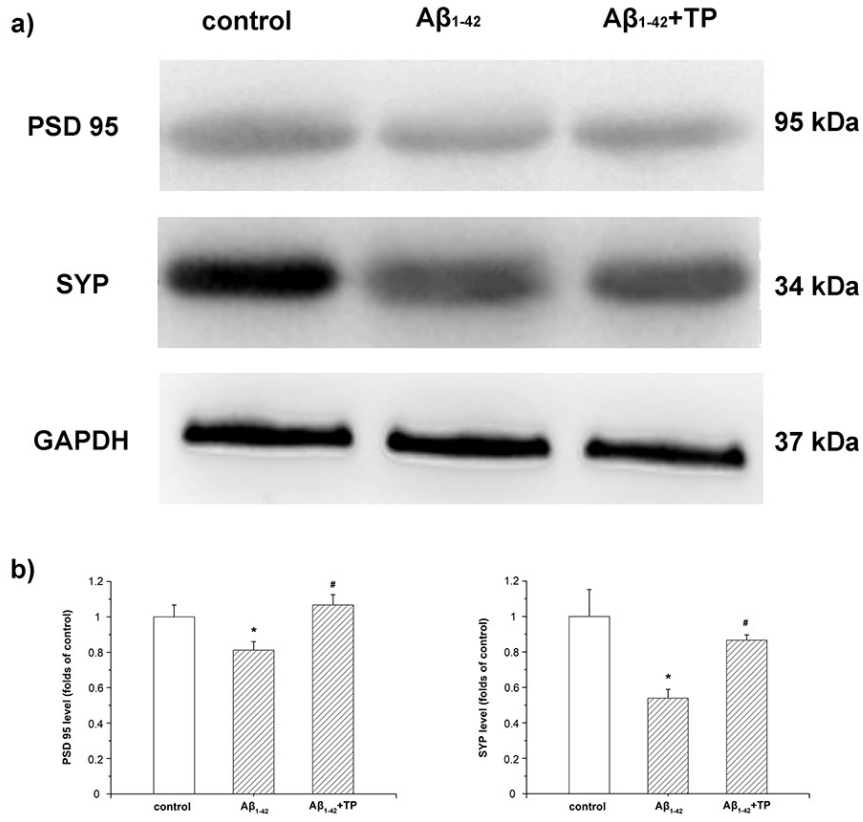

Figure 5. Effect of triptolide on the level of synaptic proteins in PC12 cells. The expression of PSD 95 and SYP in the PC12 cells was determined by Western blot, and GAPDH was used as an internal loading control. Cells were incubated with culture media, 10 μmol/L Aβ$_{1-42}$, and 10 μmol/L Aβ$_{1-42}$+10^{-9} mol/L triptolide for 24 h. **(a)** was the representative Western blot of cellular lysates. **(b)** was the corresponding linear diagram of immunoblotting quantitation. Data was calculated as the mean ±S.E.M. Each experiment was performed at least three times. $^{*}P < 0.05$ vs. control group. $^{\#}P < 0.05$ vs. 10 μmol/L Aβ$_{1-42}$ group.

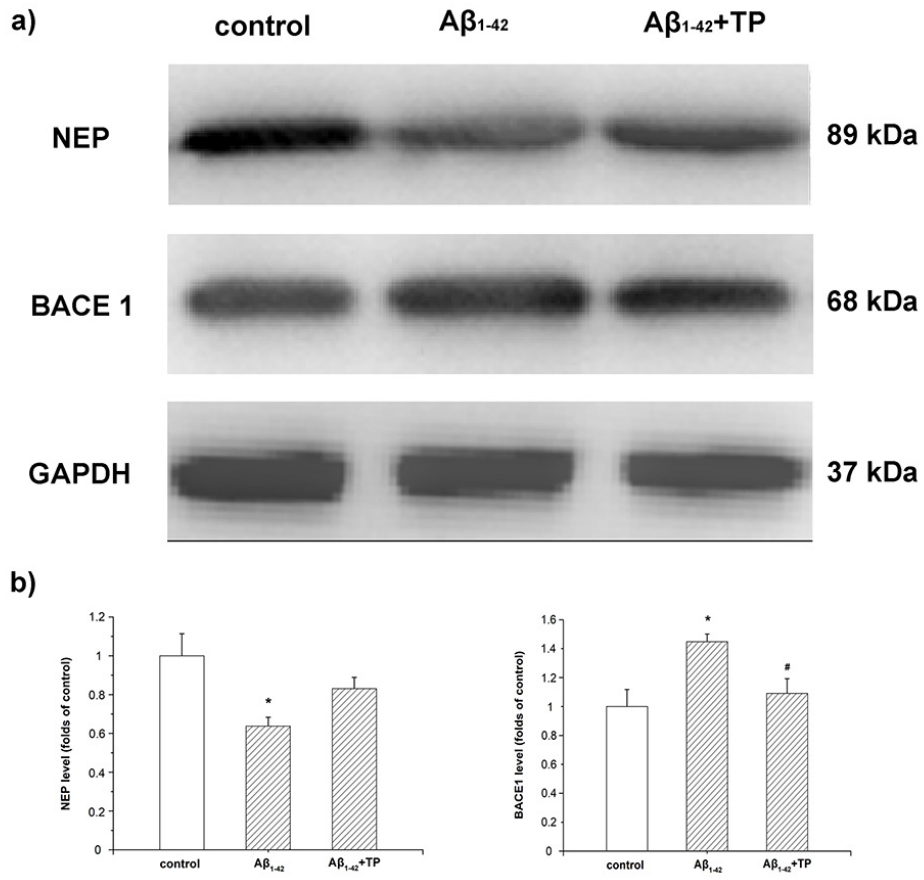

Figure 6. Effect of triptolide on Aβ generation in PC12 cells. The level of BACE1 and NEP in the PC12 cells was assessed by Western blot, and GAPDH was used as an internal loading control. Cells were incubated with a cell culture mix with the following specifications: 10 μmol/L $A\beta_{1-42}$, 10 μmol/L $A\beta_{1-42}$ and 10^{-9} mol/L triptolide for 24 h. The level of BACE1 and NEP in the PC12 cells was assessed by Western blot, and GAPDH was used as an internal loading control (a). (b) was the corresponding linear diagram of immunoblotting quantitation. Data was calculated as the mean ±S.E.M. Each experiment was conducted at least three times. *$P < 0.05$ vs. control group. #$P < 0.05$ vs. 10 μmol/L $A\beta_{1-42}$ group.

group ($P < 0.01$ on Beclin-1 and $P < 0.05$ on LC3 II/ I ratio). When incubated with triptolide, the level of Beclin-1 and the LC3 II/ I ratio decreased significantly ($P < 0.05$). The exposure of $A\beta_{1-42}$ could result in significant accumulation of p62 compared to control ($P < 0.01$), while triptolide reduced the level from 1.72 to 0.62 ($P < 0.01$), which indicates a promotion of autophagic degradation (Fig. 7b and c). Based on these results, we conclude that triptolide is able to decrease autophagosome accumulation, and induce autophagic degradation.

Triptolide treatment activated the Akt/mTOR/p70S6K pathway in PC12 cells

In the Akt/mTOR/p70S6K pathway, phosphorylated proteins are in the activated form. The results showed that $A\beta_{1-42}$ inhibited the Akt/mTOR/p70S6K pathway, and lowered the expression of p-mTOR, p-p70S6K and p-Akt compared with control group ($P < 0.05$). And triptolide could significantly reverse these results ($P < 0.01$ on p-Akt, $P < 0.05$ on p-mTOR and p-p70S6K). There were no changes in the level of total Akt, mTOR and p70S6K ($P > 0.05$). These results confirmed triptolide could activate the Akt/mTOR/p70S6K pathway in $A\beta_{1-42}$ exposed PC12 cells (Fig. 8).

Triptolide against $A\beta_{1-42}$-induced neuronal death by activating the Akt/mTOR/p70S6K pathway

When compared with the group treated with $A\beta_{1-42}$+triptolide, PC12 cell viability decreased in the Akt-I group ($P < 0.05$), the siRNA-mTOR group ($P < 0.01$), and the rapamycin group ($P < 0.01$). It is noteworthy that the siRNA-mTOR group decreased the most, while no change was

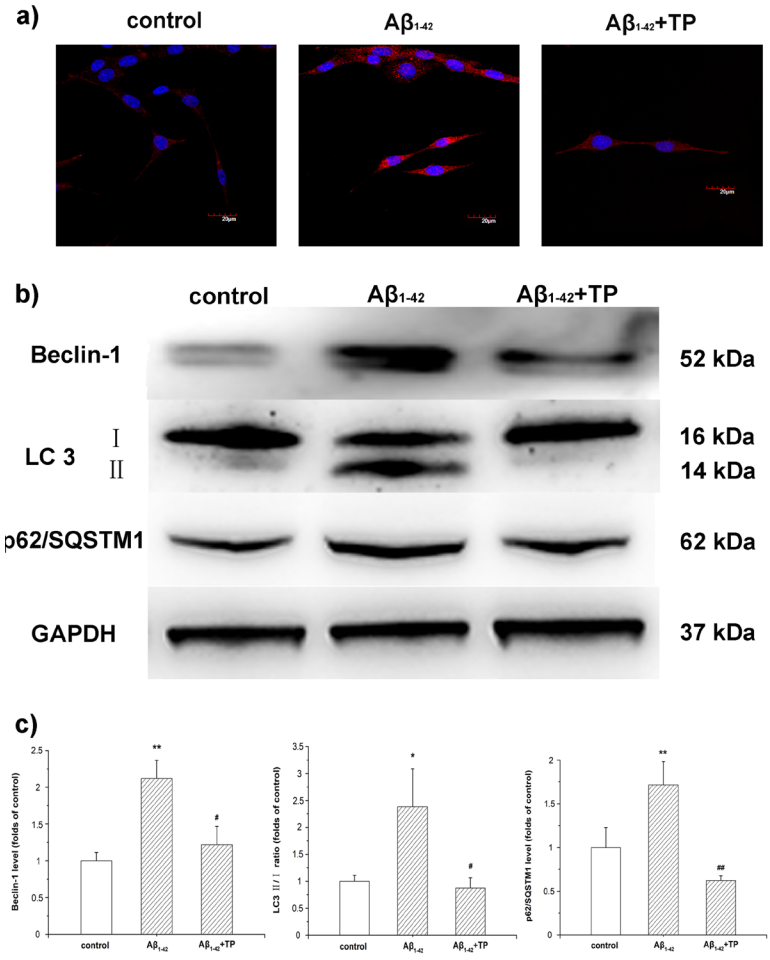

Figure 7. Effect of triptolide on $A\beta_{1-42}$-induced autophagy in PC12 cells. To characterize autophagy, the immunofluorescence of LC3 was used to indicate the autophagosome formation, and Western blot analysis was used to observe the expression of autophagy hallmark proteins Beclin-1, LC3 and p62/SQSTM1. Cells were incubated with culture media, 10 μmol/L $A\beta_{1-42}$, and 10 μmol/L $A\beta_{1-42}$+10^{-9} mol/L triptolide for 24 h. (a) was the immunofluorescence analysis of LC3 B protein in PC12 cells. LC3 B fluorescent puncta indicate autophagosome formation. (b) was the Western blotting analysis of autophagy hallmark proteins. (c) was the corresponding linear diagram of immunoblotting quantitation. Data was calculated as the mean ± S.E.M. Each experiment was conducted at least three times. *$P < 0.05$, **$P \leq 0.01$ vs. control group. #$P < 0.05$ vs. 10 μmol/L $A\beta_{1-42}$ group. #$P < 0.05$, ##$P < 0.01$ vs. 10 μmol/L Aβ1-42 group.

observed in the $A\beta_{1-42}$ group or the siRNA-mTOR group ($P > 0.05$). The result of apoptosis coincided with cell viability ($P < 0.01$ on the siRNA-mTOR group and rapamycin group, Fig. 9b and c). Our findings indicate that the neuroprotective effects of triptolide against $A\beta_{1-42}$-induced cell mortality is related to the activation of the Akt/mTOR/p70S6K mechanism.

DISCUSSION

AD is considered a global public health priority by the World Health Organization (WHO) (Lane et al. 2018). There are currently no treatments capable of preventing or treating AD, but a substantial body of evidence shows that Aβ, especially $A\beta_{1-42}$, is an important risk factor. The abnormal aggregation and fibrillation of Aβ result in the neuronal death (Zverova 2019). Previous studies have found that the survival of PC12 cells decreased after treatment with aggregated $A\beta_{25-35}$ (Xu et al. 2015, 2016). In the present study, the viability of PC12 cells was significantly lower after exposure to $A\beta_{1-42}$, and dependent on time and concentration (Fig. 1). Therefore, methods for using Aβ to slow neural mortality have generated significant attention.

Recent studies show that triptolide exhibits neuroprotection toward $A\beta_{1-42}$ toxicity (Chen et al. 2018). With the increased study of triptolide, a number of reports indicate that triptolide exerts hepatotoxicity, nephrotoxicity, cardiotoxicity, and reproductive toxicity, and its toxicity has hindered its clinical use (Gao et al. 2021). We established triptolide as safe at the beginning

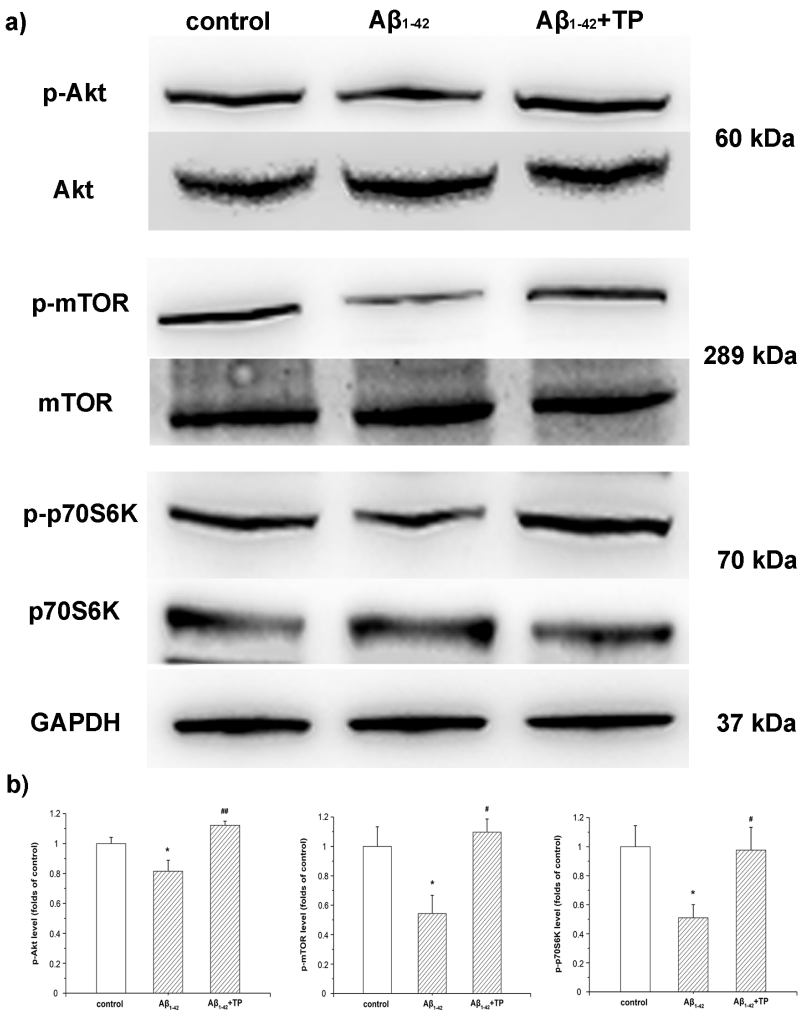

Figure 8. Effect of triptolide on the Akt/mTOR/p70S6K pathway in PC12 cells. The amount of different proteins (p-Akt, p-mTOR and p-p70S6K), which are related to the Akt/mTOR/p70S6K pathway were shown by Western blot. Cells were incubated with culture media, 10 μmol/L $A\beta_{1-42}$, and 10 μmol/L $A\beta_{1-42}$+10^{-9} mol/L triptolide for 24 h. **(a)** was the Western blotting analysis of the Akt/mTOR/p70S6K pathway hallmark proteins. The corresponding linear diagram of immunoblotting quantitation is shown in **(b)**. Data was calculated as the mean ± S.E.M. Each experiment was conducted at least three times. **P* < 0.05 vs. control group. #*P* < 0.05, ##*P* < 0.01 vs. 10 μmol/L $A\beta_{1-42}$ group.

of the experiment and observed no significant difference when compared with the control group (Fig. 2), demonstrating that these concentrations were safe for PC12 cells. Next, we measured cell viability and apoptosis to evaluate the neuroprotective effects of triptolide against $A\beta_{1-42}$ toxicity. As shown in Fig. 3 and 4, cells treated with triptolide were immune to toxicity normally induced by $A\beta_{1-42}$.

Some studies have indicated that triptolide suppresses the growth and activity of astrocytes and microglial cells in the hippocampal region, maintains cognition, and decreases neuropathology in mice with AD (Cheng et al. 2014, Li et al. 2016). Triptolide treatment reduced the expression of the β-secretase APP-cleaving enzyme 1 (BACE1) and synaptophysin expressions to protect synapses (Lu et al. 2019, Nie et al. 2012, Wang et al. 2014). Additionally, triptolide exhibited significant neuroprotection against toxicity induced by $A\beta_{1-42}$ in primary cortical neurons (Ning et al. 2018). We previously reported that triptolide directly prevents $A\beta_{25-35}$-induced neuronal apoptosis by inhibiting oxidative stress, as well as promoting PSD 95 expression in an AD cell model (Xu et al. 2015, 2016). These findings suggest that triptolide shows promise for the treatment of AD.

To further assess the effects of triptolide on neuronal function, we measured the levels of SYP and PSD 95. Studies have indicated that PSD 95 and SYP are closely related to synapse

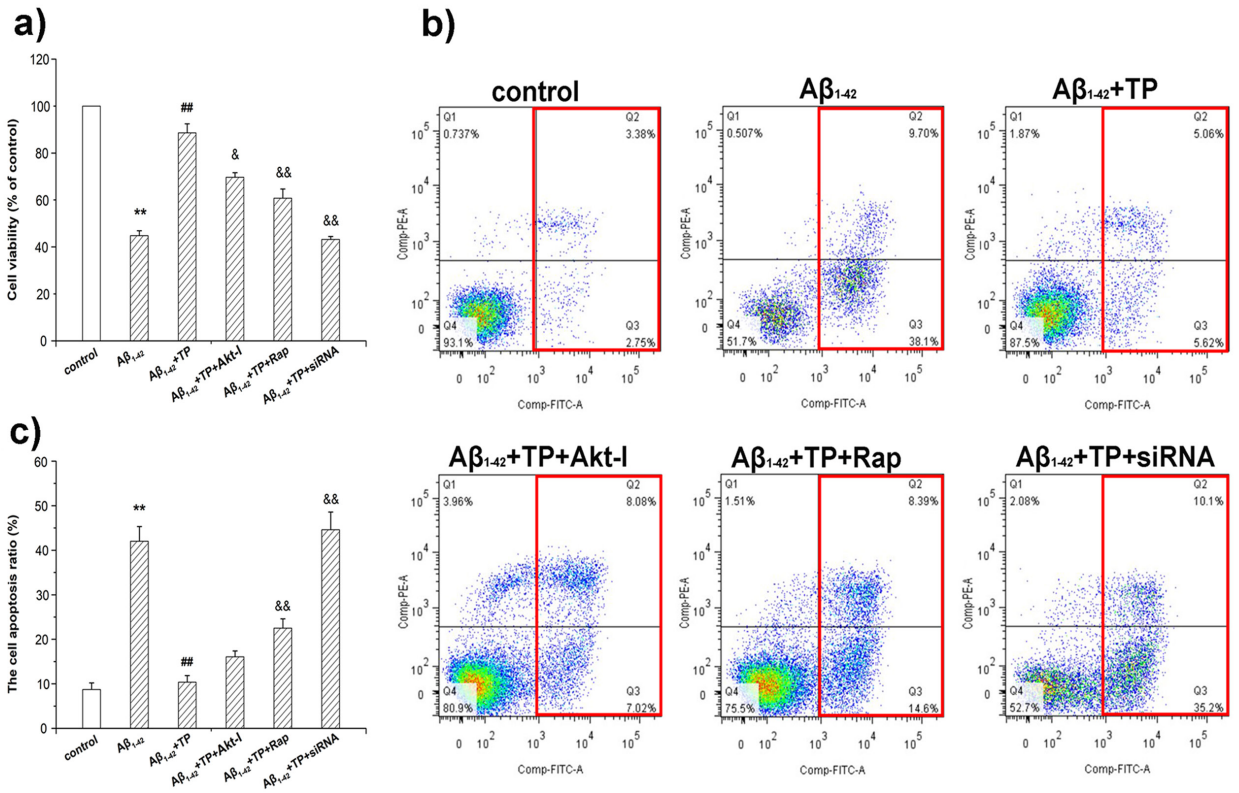

Figure 9. Effect of the Akt/mTOR/p70S6K pathway during the neuroprotection of triptolide. (a) was cell viability with CCK-8 assay. Data represent the mean ± S.E.M. n=6/group. **(b)** was flow cytometric analysis of apoptosis. The intensity of Annexin V-FITC fluorescence was on the X-axis and PI fluorescence was on the Y-axis. **(c)** was the corresponding linear diagram of flow cytometric analysis. Data represent the mean ± S.E.M. Flow cytometric assay was performed three times. A medium of cell culture (10 μmol/L $Aβ_{1-42}$ +10^{-9} mol/L triptolide, 10 μmol/L $Aβ_{1-42}$, 10 μmol/L $Aβ_{1-42}$ +10^{-9} mol/L triptolide+ 15 μmol/L Akt-I-1/2, 10 μmol/L $Aβ_{1-42}$ +10^{-9} mol/L triptolide+ 10ng/ml rapamycin, 10 μmol/L $Aβ_{1-42}$+10^{-9} mol/L triptolide+ siRNA-mTOR) were incubated for 24 h. $^{**}P < 0.01$ vs. control group. $^{\#\#}P < 0.01$ vs. 10 μmol/L $Aβ_{1-42}$ group. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ vs. 10 μmol/L $Aβ_{1-42}$+10^{-9} mol/L triptolide group.

stabilization and function in synaptic plasticity. The results indicated that the expression of PSD 95 and SYP proteins in the $Aβ_{1-42}$ group decreased significantly and treatment with triptolide enhanced the expression levels (Fig. 5). Our data demonstrated that $Aβ_{1-42}$ markedly reduced neuronal function and triptolide played an active role in promoting synaptic regeneration. This could improve or repair synaptic function in areas afflicted by AD.

BACE1, as one of the most important β-secretase enzymes, limits the rate at which enzymes catalyze the production of $Aβ_{1-42}$ (Koelsch 2017). Studies have confirmed that an increase in BACE1 levels contributes to the pathogenesis of AD. Most AD patients exhibit higher BACE1 activity in their cerebrospinal fluid compared with that of the controls, which could cause the central nervous system to overproduce $Aβ_{1-42}$ (Lopez-Font et al. 2019). Neprilysin (NEP) is the major enzyme in the brain degrading Aβ (Grimmer et al. 2019). Therefore, the inhibition of BACE1 activity and the enhancement of NEP activity could slow AD during its early stages. Recent studies have reported that triptolide inhibits the activity of β- and γ-secretase enzymes in cell and animal models of AD (Cheng et al. 2014, Wang et al. 2014). Furthermore, our results indicated that

triptolide exposure decreased BACE1 levels and increased NEP expression (Fig. 6). This indicates that a decrease in the production of $A\beta_{1-42}$ caused by triptolide may result from either a lower production of $A\beta_{1-42}$ from the processing of amyloidogenic amyloid precursor protein (APP) or an increase in the clearance of $A\beta_{1-42}$.

Autophagy plays an important role in the maintenance of healthy neurons. Yu et al. (2005) found that neuronal autophagy induced Aβ deposits in AD as well as heavy accumulation of autophagosomes in the AD cortex and hippocampus. This inhibition of autophagy can reduce the level of BACE1 (Pickford et al. 2008). Many studies have also confirmed that the inhibition of autophagy can reduce the secretion and cytotoxicity of Aβ in various cell lines and vice versa (Fan et al. 2015, Gali et al. 2019, Kuang et al. 2019).

In this study, we found that the protein levels of Beclin-1 and LC3II protein increased significantly following $A\beta_{1-42}$ treatment compared with the control group. However, treating both with triptolide resulted in suppression. Beclin-1 and LC3 II have important roles in the production and maturation of autophagosomes (Filfan et al. 2017). Beclin-1, as a marker of autophagy initiation, serves an important role in recruiting proteins involved in autophagy, whereas LC3 II is a marker of autophagy (Higgins et al. 2011, Luo et al. 2014). We also detected the expression of punctate LC3 through immunofluorescence, which has been used to monitor autophagy. Treatment with $A\beta_{1-42}$ resulted in a large number of bright fluorescent particles in cells, indicating an increased level of autophagosome formation, whereas triptolide inhibited the increase in the autophagy induced by $A\beta_{1-42}$. The results of immunofluorescence coincided with western blot results. Our results demonstrated that triptolide decreased the accumulation of autophagosomes induced by $A\beta_{1-42}$ in PC12 cells. Other studies indicate that autophagic vacuoles abnormally accumulate in the brains of AD patients or in Aβ-exposed PC12 cells, which may represent the induction of autophagy and blockage of autophagosome maturation and degradation (Mizushima 2007). Therefore, we further analyzed autophagic degradation of p62 (also known as SQSTM 1/ sequestome1). The p62 protein is selectively incorporated into autophagosomes through direct binding to LC3 and is preferentially degraded by autolysosomes. The expression of p62 is inversely correlated with autophagosome maturation and degradation. We found significant accumulation of p62 in the $A\beta_{1-42}$-treated group and triptolide decreased these levels, which indicated a promotion of autophagic degradation. Analysis of Beclin-1, LC3 II, and p62 protein degradation in triptolide-exposed cells revealed that triptolide decreased autophagosome accumulation and induced autophagic degradation.

The protein kinase B/mammalian target of the rapamysin/70 kDa ribosomal protein S6 kinase (Akt/mTOR/p70S6K) pathway is the classical autophagy pathway (Feng & Qiu 2018, Beth & Kroemer 2008). Studies indicate that the augmented activation of the Akt/mTOR/ p70S6K signaling pathway may inhibit autophagy. Furthermore, there is evidence indicating that the Akt/mTOR/p70S6K pathway is involved in Aβ-induced autophagy (O' Neill 2013, Fan et al. 2015, Maiese 2021). Therefore, we determined the effect of triptolide on the activation of the Akt/ mTOR/p70S6K pathway. A typical regulation of Akt/mTOR/p70S6K signaling has been observed in the brains of those with AD (O' Neill 2013). Fan et al. (2015) reported that autophagy induced by $A\beta_{25-35}$ inhibited the activation of the Akt/mTOR pathway in HT22 cells. Wei et al. (2012) showed that enhanced mTOR expression improved cognitive function, whereas p-p70S6K levels were significantly reduced in an AD animal model. We found that $A\beta_{1-42}$ significantly decreased

phosphorylated p70S6K, mTOR, and Akt levels, whereas triptolide upregulated expression. This indicated that the Akt/mTOR/p70S6K pathway is involved in the protection by triptolide.

Furthermore, to identify the effects of the Akt/mTOR/p70S6K signaling pathway and how it contributes to the triptolide-mediated protection of $A\beta_{1-42}$-treated PC12 cells, we inhibited the Akt/mTOR/p70S6K pathway. As shown in Fig. 9, triptolide significantly inhibited $A\beta_{1-42}$-induced cell death and apoptosis in PC12 cells and pre-treatment with an Akt inhibitor Akt-I or the mTOR inhibitor, rapamycin, reversed the protective effects of triptolide. The inhibitory effects of the siRNA-mTOR group were the most obvious. These results suggest that the reduction of autophagosome accumulation observed in triptolide-treated cells is Akt/mTOR/p70S6K pathway dependent. However, it is not clear how triptolide regulates the blocking of autophagy flux. To further assess the neuroprotective effects of triptolide on Aβ-treated differentiated PC12 cells and the pathogenesis of AD, we will continue to study the molecular signaling mechanism underlying the blockage of autophagic flux.

CONCLUSIONS

In the present study, we demonstrated for the first time that triptolide decreases autophagosome accumulation and induces autophagic degradation in Aβ-treated PC12 cells. The results indicated that activating the Akt/mTOR/p70S6K pathway induced the protective effect of triptolide. Our results provide insight into the pathogenesis of AD and reinforce triptolide as a promising therapeutic drug for treating AD. This lays the groundwork for the further development of a therapeutic strategy to treat and prevent AD in the future.

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REFERENCES

ABUBAKER AA, VARA D, VISCONTE C, EGGLESTON I, TORTI M, CANOBBIO I & PULA G. 2019. Amyloid Peptide beta1-42 Induces Integrin alphaIIbbeta3 Activation, Platelet Adhesion, and Thrombus Formation in a NADPH Oxidase-Dependent Manner. Oxid Med Cell Longev 2019: 1050476.

BALDASSARRE M, BARONIO CM, MOROZOVA-ROCHE LA & BARTH A. 2017. Amyloid beta-peptides 1-40 and 1-42 form oligomers with mixed beta-sheets. Chem Sci 8: 8247-8254.

BETH L & KROEMER G. 2008. Autophagy in the pathogenesis of disease. Cell 132: 27-42.

CHAN SF, CHEN YY, LIN JJ, LIAO CL, KO YC, TANG NY, KUO CL, LIU KC & CHUNG JG. 2017. Triptolide induced cell death through apoptosis and autophagy in murine leukemia WEHI-3 cells in vitro and promoting immune responses in WEHI-3 generated leukemia mice in vivo. Environ Toxicol 32: 550-568.

CHEN SR, DAI Y, ZHAO J, LIN L, WANG Y & WANG Y. 2018. A Mechanistic Overview of Triptolide and Celastrol, Natural Products from Tripterygium wilfordii Hook F. Front Pharmacol 9: 104.

CHENG S, LEBLANC KJ & LI L. 2014. Triptolide preserves cognitive function and reduces neuropathology in a mouse model of Alzheimer's disease. PLoS ONE 9: e108845.

CHENG W, CHEN W, WANG P & CHU J. 2018. Asiatic acid protects differentiated PC12 cells from Abeta25-35-induced apoptosis and tau hyperphosphorylation via regulating PI3K/Akt/GSK-3beta signaling. Life Sci 208: 96-101.

FABIANI C & ANTOLLINI SS. 2019. Alzheimer's Disease as a Membrane Disorder: Spatial Cross-Talk Among Beta-Amyloid Peptides, Nicotinic Acetylcholine Receptors and Lipid Rafts. Front Cell Neurosci 13: 309.

FAN S, ZHANG B, LUAN P, GU B, WAN Q, HUANG X, LIAO W & LIU J. 2015. PI3K/AKT/mTOR/p70S6K Pathway Is Involved in Abeta25-35-Induced Autophagy. Biomed Res Int 2015: 161020.

FENG FB & QIU HY. 2018. Effects of Artesunate on chondrocyte proliferation, apoptosis and autophagy through the PI3K/AKT/mTOR signaling pathway in rat models with rheumatoid arthritis. Biomed Pharmacother 102: 1209-1220.

FILFAN M, SANDU RE, ZAVALEANU AD, GRESITA A, GLAVAN DG, OLARU DG & POPA-WAGNER A. 2017. Autophagy in aging and disease. Rom J Morphol Embryol 58: 27-31.

GALI CC ET AL. 2019. Amyloid-beta impairs insulin signaling by accelerating autophagy-lysosomal degradation of LRP-1 and IR-beta in blood-brain barrier endothelial cells in vitro and in 3XTg-AD mice. Mol Cell Neurosci 99: 103390.

GAO J, ZHANG Y, LIU X, WU X, HUANG L & GAO W. 2021. Triptolide: pharmacological spectrum, biosynthesis, chemical synthesis and derivatives. Theranostics 11: 7199-7221.

GAO L, ZHOU F, WANG KX, ZHOU YZ, DU GH & QIN XM. 2020. Baicalein protects PC12 cells from Abeta25-35-induced cytotoxicity via inhibition of apoptosis and metabolic disorders. Life Sci 248: 117471.

GRIMMER T, GOLDHARDT O, YAKUSHEV I, ORTNER M, SORG C, DIEHL-SCHMID J, FORSTL H, KURZ A, PERNECZKY R & MINERS S. 2019. Associations of Neprilysin Activity in CSF with Biomarkers for Alzheimer's Disease. Neurodegener Dis 19(1): 43-50.

HADIPOUR E, TAYARANI-NAJARAN Z & FEREIDONI M. 2020. Vitamin K2 protects PC12 cells against Abeta (1-42) and H2O2-induced apoptosis via p38 MAP kinase pathway. Nutr Neurosci 23: 343-352.

HIGGINS GC, DEVENISH RJ, BEART PM & NAGLEY P. 2011. Autophagic activity in cortical neurons under acute oxidative stress directly contributes to cell death. Cell Mol Life Sci 68(22): 3725-3740.

ITOH N, TAKADA E, OKUBO K, YANO Y, HOSHINO M, SASAKI A, KINJO M & MATSUZAKI K. 2018. Not Oligomers but Amyloids are Cytotoxic in the Membrane-Mediated Amyloidogenesis of Amyloid-beta Peptides. Chembiochem 19: 430-433.

JESKO H, LUKIW WJ, WILKANIEC A, CIESLIK M, GASSOWSKA-DOBROWOLSKA M, MURAWSKA E, HILGIER W & ADAMCZYK A. 2018. Altered Expression of Urea Cycle Enzymes in Amyloid-beta Protein Precursor Overexpressing PC12 Cells and in Sporadic Alzheimer's Disease Brain. J Alzheimers Dis 62(1): 279-291.

JIANG S, ZHAO Y, ZHANG T, LAN J, YANG J, YUAN L, ZHANG Q, PAN K & ZHANG K. 2018. Galantamine inhibits beta-amyloid-induced cytostatic autophagy in PC12 cells through decreasing ROS production. Cell Prolif 51(3): e12427.

KNOPMAN DS, JONES DT & GREICIUS MD. 2021. Failure to demonstrate efficacy of aducanumab: An analysis of the EMERGE and ENGAGE trials as reported by Biogen, December 2019. Alzheimers Dement 17: 696-701.

KOELSCH G. 2017. BACE1 Function and Inhibition: Implications of Intervention in the Amyloid Pathway of Alzheimer's Disease Pathology. Molecules 22(10): 1723.

KUANG H, TAN CY, TIAN HZ, LIU LH, YANG MW, HONG FF & YANG SL. 2019. Exploring the bi-directional relationship between autophagy and Alzheimer's disease. CNS Neurosci Ther 26(2): 155-166.

LANE CA, HARDY J & SCHOTT JM. 2018. Alzheimer's disease. Eur J Neurol 25: 59-70.

LI DD, ZHANG YH, ZHANG W & ZHAO P. 2019. Meta-Analysis of Randomized Controlled Trials on the Efficacy and Safety of Donepezil, Galantamine, Rivastigmine, and Memantine for the Treatment of Alzheimer's Disease. Front Neurosci 13: 472.

LI J & HAO J. 2019. Treatment of Neurodegenerative Diseases with Bioactive Components of Tripterygium wilfordii. Am J Chin Med 47: 769-785.

LI JM, ZHANG Y, TANG L, CHEN YH, GAO Q, BAO MH, XIANG J & LEI DL. 2016. Effects of triptolide on hippocampal microglial cells and astrocytes in the APP/PS1 double transgenic mouse model of Alzheimer's disease. Neural Regen Res 11: 1492-1498.

LI Q, LIU Y & SUN M. 2017a. Autophagy and Alzheimer's Disease. Cell Mol Neurobiol 37: 377-388.

LI XY, WANG SS, HAN Z, HAN F, CHANG YP, YANG Y, XUE M, SUN B & CHEN LM. 2017b. Triptolide Restores Autophagy to Alleviate Diabetic Renal Fibrosis through the miR-141-3p/PTEN/Akt/mTOR Pathway. Mol Ther Nucleic Acids 9: 48-56.

LIU JY ET AL. 2018. Downregulation of Aquaporin 9 Exacerbates Beta-amyloid-induced Neurotoxicity in Alzheimer's Disease Models In vitro and In vivo. Neuroscience 394: 72-82.

LOPEZ-FONT I, BOIX CP, ZETTERBERG H, BLENNOW K & SAEZ-VALERO J. 2019. Characterization of Cerebrospinal Fluid BACE1 Species. Mol Neurobiol 56(12): 8603-8616.

LU X, YANG B, YU H, HU X, NIE J, WAN B, ZHANG M & LU C. 2019. Epigenetic mechanisms underlying the effects of triptolide and tripchlorolide on the expression of neuroligin-1 in the hippocampus of APP/PS1 transgenic mice. Pharm Biol 57: 453-459.

LUO T, LIU G, MA H, LU B, XU H, WANG Y, WU J, GE P & LIANG J. 2014. Inhibition of autophagy via activation of PI3K/Akt pathway contributes to the protection of ginsenoside Rb1 against neuronal death caused by ischemic insults. Int J Mol Sci 15: 15426-15442.

MAIESE K. 2021. Targeting the core of neurodegeneration: FoxO, mTOR, and SIRT1. Neural Regen Res 16: 448-455.

MIAO H, MIAO C, HAN J & LI N. 2020. Downregulation of miR-200a Protects Mouse Leydig Cells Against Triptolide by Triggering Autophagy. Drug Des Devel Ther 14: 4845-4854.

MIZUSHIMA N. 2007. Autophagy: process and function. Genes Dev 21: 2861-2873.

NIE J, ZHOU M, LU C, HU X, WAN B, YANG B & LI Y. 2012. Effects of triptolide on the synaptophysin expression of hippocampal neurons in the AD cellular model. Int Immunopharmacol 13: 175-180.

NING C, MO L, CHEN X, TU W, WU J, HOU S & XU J. 2018. Triptolide derivatives as potential multifunctional anti-Alzheimer agents: Synthesis and structure-activity relationship studies. Bioorg Med Chem Lett 28: 689-693.

O' NEILL C. 2013. PI3-kinase/Akt/mTOR signaling: impaired on/off switches in aging, cognitive decline and Alzheimer's disease. Exp Gerontol 48: 647-653.

PICKFORD F ET AL. 2008. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta accumulation in mice. J Clin Invest 118: 2190-2199.

SCHELTENS P, BLENNOW K, BRETELER MM, DE STROOPER B, FRISONI GB, SALLOWAY S & VAN DER FLIER WM. 2016. Alzheimer's disease. Lancet 388: 505-517.

SELKOE DJ. 2001. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81: 741-766.

SINYOR B, MINEO J & OCHNER C. 2020. Alzheimer's Disease, Inflammation, and the Role of Antioxidants. J Alzheimers Dis Rep 4: 175-183.

SORIA LOPEZ JA, GONZALEZ HM & LEGER GC. 2019. Alzheimer's disease. Handb Clin Neurol 167: 231-255.

WANG Q ET AL. 2014. Triptolide treatment reduces Alzheimer's disease (AD)-like pathology through inhibition of BACE1 in a transgenic mouse model of AD. Dis Model Mech 7: 1385-1395.

WEI L ET AL. 2012. A butyrolactone derivative 3BDO alleviates memory deficits and reduces amyloid-beta deposition in an AbetaPP/PS1 transgenic mouse model. J Alzheimers Dis 30: 531-543.

XIE C, JIANG J, LIU J, YUAN G & ZHAO Z. 2019. Triptolide suppresses human synoviocyte MH7A cells mobility and maintains redox balance by inhibiting autophagy. Biomed Pharmacother 115: 108911.

XU P, LI Z, WANG H, ZHANG X & YANG Z. 2015. Triptolide Inhibited Cytotoxicity of Differentiated PC12 Cells Induced by Amyloid-Beta(2)(5)(-)(3)(5) via the Autophagy Pathway. PLoS ONE 10: e0142719.

XU P, WANG H, LI Z & YANG Z. 2016. Triptolide attenuated injury via inhibiting oxidative stress in Amyloid-Beta25-35-treated differentiated PC12 cells. Life Sci 145: 19-26.

YANG P, JIN J, LIU Q, MA D, LI J, ZHANG Y & LIU Y. 2019. Optimization of Degradation Conditions with PRG, a Polysaccharide from Phellinus ribis, by RSM and the Neuroprotective Activity in PC12 Cells Damaged by Abeta25-35. Molecules 24(16): 3010.

YU WH ET AL. 2005. Macroautophagy--a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol 171: 87-98.

ZHANG R, ZHENG Y, HU F, MENG X, LV B, LAO K, GAO X, ZHANG X & GOU X. 2020. Effect of (m)VD-hemopressin against Abeta1-42-induced oxidative stress and apoptosis in mouse hippocampal neurons. Peptides 124: 170185.

ZVEROVA M. 2019. Clinical aspects of Alzheimer's disease. Clin Biochem 72: 3-6.

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