Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Tanshinone IIA Alleviates Amyloid β-Induced Neurotoxicity of SH-SY5Y Cells through GSK-3β Pathway

Rongrong Huang, Songxia Lu

Department of Pharmacology, Anhui Xinhua University, Hefei, Anhui Province, China

Submitted: 12-Jun-2020 Revised: 06-Jul-2020 Accepted: 09-Mar-2021 Published: 11-Nov-2021

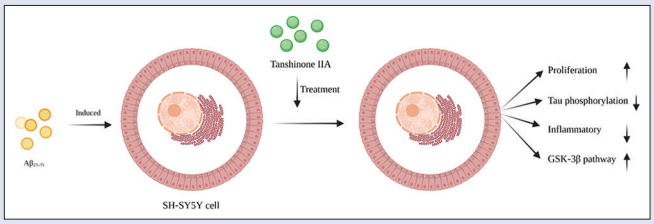
ABSTRACT

Background: The deposition of amyloid β (Aβ) proteins and hyperphosphorylation of tau proteins are the two notable features of Alzheimer's disease. During the past decades, novel drug candidates have been found from natural herbs and its derived compounds due to their broad spectra of therapeutic effects with low toxicity. Among the different compounds studied, tanshinone IIA, which is derived from *Salvia miltiorrhiza*, has been reported to attenuate Aβ-induced neurotoxicity. **Materials and Methods:** In this study, we studied the effects of tanshinone IIA on the neurotoxicity, proliferation, and apoptosis of $Aβ_{25–35}$-induced SH-SY5Y cells. We applied various methods such as Western blot, fluorescence staining, and flow cytometry. We analyzed the tau phosphorylation and inflammatory response of SH-SY5Y cells, and we further discuss the relationship between phosphorylated tau and GSK-3β pathway. **Results:** Tanshinone IIA promoted proliferation and inhibited neurotoxicity of $Aβ_{25–35}$-induced SH-SY5Y cells. In addition, it downregulated the level of phosphorylation of tau protein, leading to the inhibition of inflammatory response. The Y216 phosphorylation level of GSK-3β was downregulated by tanshinone IIA, whereas the S9 phosphorylation level was upregulated. **Conclusion:** The results of this study provide evidence that tanshinone IIA exerts its beneficial effects by attenuating the neurotoxicity induced by $Aβ_{25–35}$ through the GSK-3β pathway.

Key words: Alzheimer's disease, amyloid β peptide, GSK-3β pathway, SH-SY5Y cells, tanshinone IIA, tau phosphorylation

SUMMARY

- Tanshinone IIA promoted proliferation and inhibited neurotoxicity of $Aβ_{25–35}$-induced SH-SY5Y cells. In addition, it downregulated the level of phosphorylation of tau protein, leading to the inhibition of inflammatory response. The Y216 phosphorylation level of GSK-3β was downregulated by tanshinone IIA, whereas the S9 phosphorylation level was upregulated.



Abbreviations used: Aβ: The deposition of amyloid β; AD: Alzheimer's disease

Correspondence:

Prof. Rongrong Huang,
Department of Pharmacology, Anhui Xinhua University, No. 555 Wangjiang West Road, Hefei, Anhui Province 230088, China.
E-mail: huangrongrong362@126.com

DOI: 10.4103/pm.pm_249_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Alzheimer's disease (AD), the most common type of senile dementia, has several pathological features, including neuronal degeneration, intracellular neurofibrillary tangles, and extracellular senile plaque deposition.[1] Although a large number of investigators have been searching for effective methods for the diagnosis and treatment of this disease, the pathogenesis of AD is still not clear. In the pathological process known so far, the dynamic balance between the production and clearance of amyloid β (Aβ) peptides is destabilized, resulting in the abnormal accumulation of Aβ peptides. The complex cascade of reactions triggers multiple processes, including plaque formation, tau protein hyperphosphorylation, glial cell proliferation, and inflammation, which results in nerve cell dysfunction.[2] Therefore, Aβ is a promising target in the treatment of AD, which has been widely investigated.[3,4]

During the past few decades, great efforts have been made to develop drugs for the prevention and treatment of AD. So far, numerous drug candidates have been studied, such as natural compounds derived from herbs.[5-7] Among these, tanshinone IIA [Figure 1a], a diterpenoid compound isolated from *Salvia miltiorrhiza*, is widely used in the treatment of cardiovascular diseases. This compound shows antiapoptosis, anti-inflammation, and antioxidant activity.[8,9] Recently, several studies have revealed their positive effects of tanshinone IIA on diseases affecting the nervous system, demonstrating the therapeutic potential of tanshinone IIA in preventing and alleviating AD. For example, Qian *et al.*[10] demonstrated that tanshinone IIA protects neurons against Aβ-induced neuronal injury through the activation of the Bcl-xL pathway. Jiang *et al.*[11] found that tanshinone IIA decreases the release of nitric oxide, peroxynitrite, and anion, as well as the expression of inducible nitric oxide synthase, matrix metalloprotein type 2, and nuclear factor-kappa B in the AD rat model. In a recent study, Geng *et al.*[12] reported that tanshinone IIA reduced the level of Aβ-induced neurotoxicity due to its superior antioxidant properties.

Cite this article as: Huang R, Lu S. Tanshinone IIA alleviates amyloid β-induced neurotoxicity of SH-SY5Y cells through GSK-3β pathway. Phcog Mag 2021;17:552-7.

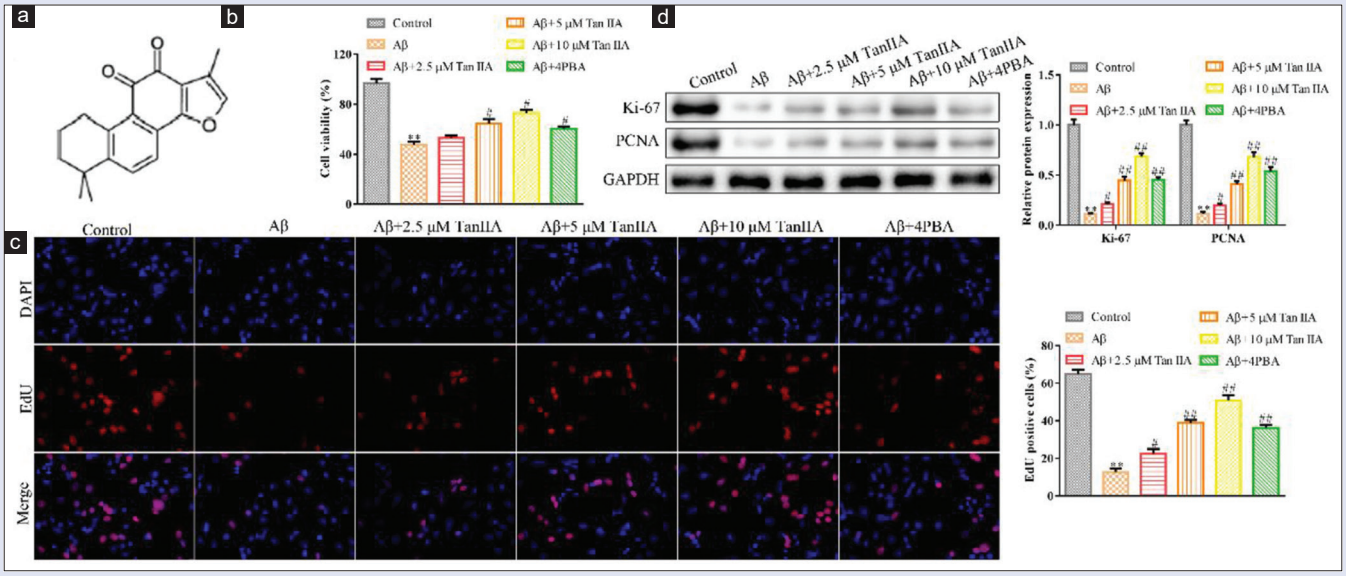


Figure 1: Effects of tanshinone IIA on the proliferation of SH-SY5Y cells injured by $A\beta_{25-35}$. (a) Chemical structure of tanshinone IIA. After treating with sodium 4-phenylbutyrate (5 mM) or tanshinone IIA (2.5 μM, 5 μM, and 10 μM), the proliferation of $A\beta_{25-35}$-injured SH-SY5Y cells was measure by (b) CCK8 assay, (c) EdU staining, and (d) Western blot for cell proliferation factor (Ki-67 and PCNA). Statistical data are presented as mean ± standard deviation $^{**}P < 0.01$, versus control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus Aβ group. The experiments were independently repeated three times

In this study, the neuroprotective effects of tanshinone IIA on Aβ-induced SH-SY5Y cells were investigated. The proliferation of Aβ-induced SH-SY5Y cells was found to be promoted by tanshinone IIA. In addition, the apoptosis of Aβ-induced SH-SY5Y cells was inhibited. Moreover, we demonstrated that tanshinone IIA downregulated the phosphorylation level of tau protein in these cells, leading to the inhibition of the inflammatory response. GSK-3β pathway, which could cause hyperphosphorylation tau, was further demonstrated to be regulated corresponding to the tanshinone IIA treatment. Our research may provide proof-of-principle evidence of the mechanism through which tanshinone IIA prevents Aβ-induced neurotoxicity of SH-SY5Y cells.

MATERIALS AND METHODS

Compounds

Tanshinone IIA ($C_{19}H_{18}O_3$, FW: 294.34, purity >98%) was obtained from MedChemExpress (Shanghai, China). $A\beta_{25-35}$ peptide (purity >95%) was purchased from GL Biochem Inc. (Shanghai, China). Sodium 4-phenylbutyrate (4-PBA) ($C_{10}H_{12}O_2$, FW: 164.20, purity >98%) was obtained from Sigma-Aldrich (Shanghai, China).

Cell treatment

For the establishment of $A\beta_{25-35}$-induced SH-SY5Y cell model, $A\beta_{25-35}$ was diluted to 1 mM by distilled water for a stock solution and then cultured in a 37°C incubator for 3 days to aggregate oligomers before use The activated $A\beta_{25-35}$ was diluted to 25 μM and stored at −20°C. Cells (1×10^5) were seeded in a 6-well plate (1 mL) and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). When the confluency reached 70%, the medium was replaced with a serum-free culture medium and incubated for 24 h. Then, $A\beta_{25-35}$ (25 μM) was added and incubated for another 24 h for use in subsequent experiments. To understand the role of tanshinone IIA, the experimental groups were set up, including a blank control group, the negative control group (phosphate-buffered saline [PBS]), tanshinone IIA (2.5, 5, and 10 μM) experimental group, and positive control group (4-PBA). The effects of 4-PBA were determined by treating the cells with 5 mM 4-PBA.

Cell viability analysis

Cells (5×10^3) were seeded in 96-well plate (100 μL) and cultured in RPMI 1640 medium supplemented with 10% FBS. When the confluency reached 70%, the medium was replaced with a medium containing different concentrations of tanshinone IIA. After 24 h of incubation, 20 μL CCK-8 solution (Beyotime Biotechnology, Nantong, China) was added to each well. After incubating for 1 h, the absorbance was detected at 450 nm by a microplate reader (Thermo Fisher, Massachusetts, USA).

Quantitative real-time polymerase chain reaction

SH-SY5Y cells (1×10^5) were seeded in a 6-well plate (1 mL) and treated according to the experimental group. When the confluency reached 90%, the RNA extraction was performed by TRIzol reagent as described previously.[13] The concentration of RNA was measured by spectrophotometer, and all samples were balanced by reverse transcription with a cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany). After the implementation of a real-time reverse-transcription polymerase chain reaction, the cycle threshold (CT) value of the target gene expression was obtained and compared with the control group. *GAPDH* was used as the internal control gene. The relative quantitative analysis was conducted by $2^{-\Delta\Delta CT}$ method.

EdU staining

SH-SY5Y cells (1×10^5) were seeded in a 6-well plate (1 mL) and treated according to the experimental group. After treatment, the cells were fixed with 4% paraformaldehyde and permeated with 0.5% Triton X-100. After washing (thrice) with PBS containing 3% BSA, the EdU staining solution (US Everbright, Suzhou, China) was added and incubated at room temperature (RT) for 30 min. Subsequently, the nucleus was counterstained with DAPI, and the cells were observed with a fluorescence microscope (IX73, Olympus, Japan).

Cell apoptosis analysis

Cells (1×10^5) were seeded in a 6-well plate (1 mL) and cultured for 24 h. After the treatment of the cells as mentioned above, the cells were

collected (×1000 rpm for 5 min) and washed with PBS. Then, the cells were resuspended with 5 μL Annexin V-fluorescein isothiocyanate (FITC) and 5 μL of propidium iodide (PI) and incubated at RT for 15 min. Flow cytometric analysis was conducted by a FACSCalibur flow cytometer (BD Bioscience, New Jersey, USA).

TUNEL analysis

Cells (1×10^5) were seeded in a 6-well plate (1 mL) and treated according to the experimental group. After treatment, the cells were fixed with 4% paraformaldehyde and permeated with 0.5% Triton. After washing (thrice) with PBS containing 3% BSA, cells were treated with TUNEL staining. The experiment was conducted according to the instructions of the TUNEL Assay Kit (Abcam, Shanghai, China). Finally, the nuclei were counterstained with DAPI, and the cells were observed with a fluorescence microscope (IX73, Olympus, Japan).

Enzyme-linked immunosorbent assay

The inflammatory factors in the supernatant of the cell culture medium were detected according to the instructions. All enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Shanghai, China). Finally, the value of optical density for each well was measured by a microplate reader at 450 nm wavelength (Thermo Fisher, Massachusetts, USA).

Immunofluorescence analysis

The cells were inoculated into a Petri dish with pretreated glass slides. When the confluency reached 80%, the cells were fixed with 4% paraformaldehyde and permeated with 0.5% Triton. Subsequently, the cells were washed (thrice) with PBS and blocked with 3% (w/v) BSA at 4°C overnight. Then, the cell was incubated with primary antibodies against p-GSK-3β-Y216 (1:500 dilution) and p-GSK-3β-S9 (1:500 dilution) at 4°C overnight. All antibodies were purchased from Abcam (Shanghai, China). After incubating with FITC-conjugated secondary antibodies (1:200 dilution, Proteintech, Wuhan, China) at RT for 1 h, the slides were observed with a fluorescence microscope (IX73, Olympus, Japan).

Statistical analysis

Data were presented in the form of mean ± standard deviation. Statistical analysis was performed by GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA), and differences between groups were analyzed by one-way analysis of variance and Dunnett's multiple comparison tests. A $P < 0.05$ was considered statistically significant.

RESULTS

Tanshinone IIA promotes the proliferation of amyloid β_{25-35}-induced SH-SY5Y cells

In this study, we studied the effects of tanshinone IIA on the proliferation of SH-SY5Y cells. As revealed in Figure 1b, the viability of SH-SY5Y cells was significantly inhibited, indicating that the cell model was successfully established. These results also suggested that 4-PBA (an inhibitor of endoplasmic reticulum stress) significantly attenuated the $A\beta_{25-35}$-induced neurotoxicity [$P < 0.05$, vs. Aβ group, Figure 1b]. Of note, both 5 μM and 10 μM tanshinone IIA significantly attenuated the $A\beta_{25-35}$-induced neurotoxicity [$P < 0.05$, vs. Aβ group, Figure 1b]. Moreover, EdU staining was also employed to analyze the proliferation of $A\beta_{25-35}$-induced SH-SY5Y cells. The results suggested that tanshinone IIA significantly promoted the proliferation of Aβ-induced SH-SY5Y cells [Figure 1c]. Moreover, tanshinone IIA promoted the proliferation of SH-SY5Y cells. Next, we investigated the expression of the cell proliferation factor of $A\beta_{25-35}$-induced SH-SY5Y cells. As demonstrated in Figure 1d, tanshinone IIA significantly increased the expression levels of Ki-67 and PCNA [Figure 1d]. In addition, tanshinone IIA exhibited comparable effects with 4-PBA on the proliferation of $A\beta_{25-35}$-induced SH-SY5Y cells. Overall, our results show that tanshinone IIA promotes the proliferation of $A\beta_{25-35}$-induced SH-SY5Y cells.

Tanshinone IIA rescued amyloid β_{25-35}-induced apoptosis of SH-SY5Y cells

In this study, we studied the effects of tanshinone IIA on the apoptosis of SH-SY5Y cells using TUNEL analysis. According to our results, DNA fragments with 3'-OH cohesive terminus of SH-SY5Y cells were in increased quantities in response to Aβ treatment, which indicates that the apoptosis of $A\beta_{25-35}$-induced SH-SY5Y cells was upregulated. In contrast, tanshinone IIA also decreased the DNA fragments in $A\beta_{25-35}$-induced SH-SY5Y cells [Figure 2a]. In addition, the results of Annexin V/PI staining suggested that tanshinone IIA significantly decreased the level of apoptosis [$P < 0.05$, vs. Aβ group, Figure 2b]. In this study, we analyzed the apoptotic pathway via Western blot analysis. According to the results of Western blot analysis, tanshinone IIA downregulated the expression of Bax and upregulated the expression of Bcl-2 proteins [$P < 0.01$, vs. Aβ group, Figure 2c]. Moreover, it exhibited comparable effects with 4-PBA on the apoptosis of $A\beta_{25-35}$-induced SH-SY5Y cells. Overall, these data indicate that tanshinone IIA rescued apoptosis in $A\beta_{25-35}$-induced SH-SY5Y cells.

Tanshinone IIA inhibits tau phosphorylation and inflammatory response in amyloid β_{25-35}-induced SH-SY5Y cells

Next, we analyzed the level of tau phosphorylation via Western blot analysis. According to our results, the phosphorylation of tau at Ser205, Ser396, and Ser413 was all significantly upregulated by $A\beta_{25-35}$ peptides [$P < 0.01$, vs. control group, Figure 3a], and tanshinone IIA remarkably inhibited the phosphorylation of tau at these sites. These results show that tanshinone IIA downregulated the $A\beta_{25-35}$-induced neuroinflammation in SH-SY5Y cells. Hence, the expression of inflammatory cytokines was further analyzed by ELISA and qPCR. As revealed in Figure 3b and c, the protein and mRNA levels of inflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin [IL]-6, IL-1β, and IL-10) were upregulated by $A\beta_{25-35}$ peptides ($P < 0.01$, vs. control group). Moreover, tanshinone IIA decreased the secretion of the aforementioned inflammatory cytokines from the $A\beta_{25-35}$-induced SH-SY5Y cells [Figure 3b]. In addition, the mRNA level of inflammatory factors was also markedly downregulated by tanshinone IIA [$P < 0.05$, vs. Aβ group, Figure 3c]. Overall, tanshinone IIA exhibited comparable effects with 4-PBA on the tau phosphorylation and inflammation in the $A\beta_{25-35}$-induced SH-SY5Y cells. These results indicated that tanshinone IIA inhibited the phosphorylation level of tau, leading to the downregulation of the Aβ-induced neuroinflammation.

Tanshinone IIA regulates GSK-3β pathway of amyloid β-induced SH-SY5Y cells

In this study, we investigated the activation of GSK-3β pathway, which could cause hyperphosphorylation of tau. First, the fluorescence intensity of p-GSK-3β-Y216 was increased after inducing the cells with $A\beta_{25-35}$ peptides; however, the fluorescence intensity of p-GSK-3β-S9 was decreased [Figure 4a]. Furthermore, our results show that the expression of p-GSK-3β-Y216 was downregulated in $A\beta_{25-35}$-induced SH-SY5Y cells by tanshinone IIA, whereas the intensity of p-GSK-3β-S9 was increased [Figure 4a]. Our findings show that tanshinone IIA might

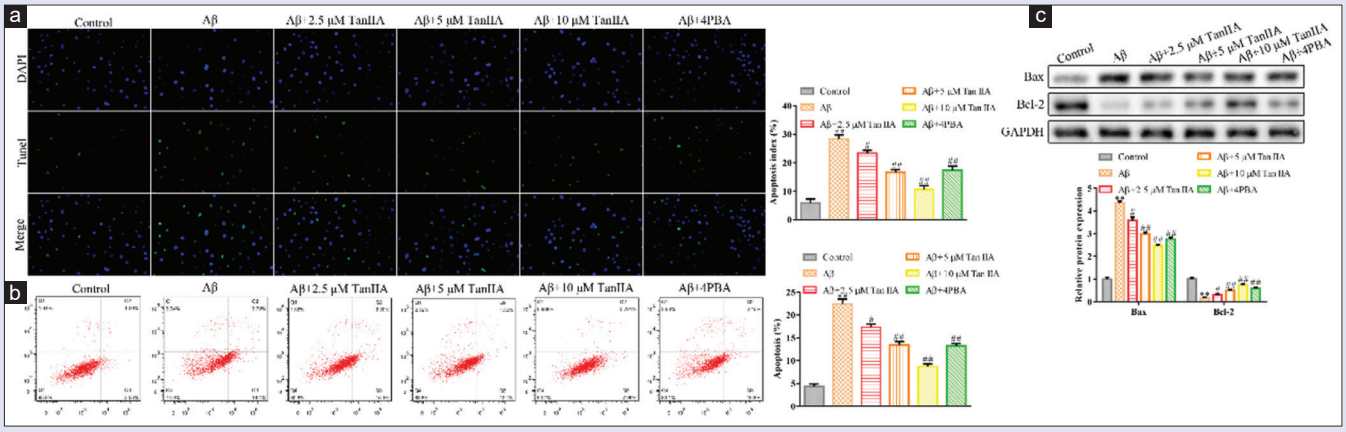


Figure 2: Effects of tanshinone IIA on the apoptosis of SH-SY5Y cells injured by $A\beta_{25-35}$. After treating with sodium 4-phenylbutyrate (5 mM) or indicated concentrations of tanshinone IIA, the apoptosis of $A\beta_{25-35}$-injured SH-SY5Y cells was measure by (a) TUNEL assay, (b) flow cytometry with Annexin V/PI Staining and (c) Western Blot for apoptosis pathway, including Bax and Bcl-2. Statistical data are presented as mean ± standard deviation $^{**}P < 0.01$, versus control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus Aβ group. The experiments were independently repeated three times

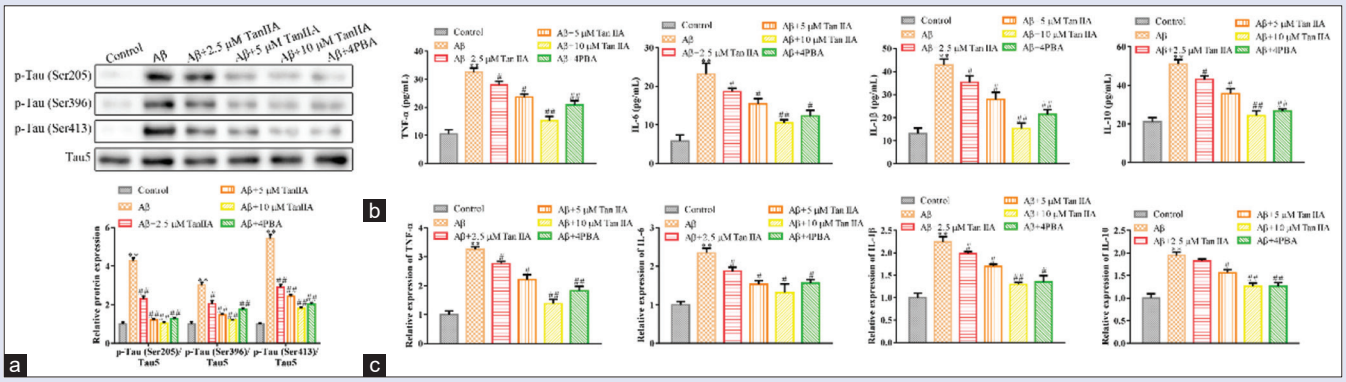


Figure 3: Effects of tanshinone IIA on phosphorylation of tau protein and inflammatory factors expression of SH-SY5Y cells injured by $A\beta_{25-35}$. After treating with sodium 4-phenylbutyrate (5 mM) or indicated concentrations of tanshinone IIA on $A\beta_{25-35}$-injured SH-SY5Y cells, (a) protein levels of phosphorylated tau proteins (Ser205, Ser396, and Ser413), and total tau protein (Tau-5) were measured by Western blot, (b) expression of inflammatory factors (TNF-α, IL-6, IL-1β, and IL-10) was measured by ELISA, and (c) mRNA expression levels of inflammatory factors (TNF-α, IL-6, IL-1β, and IL-10) were measured by qPCR. Statistical data are presented as mean ± standard deviation $^{**}P < 0.01$, versus control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus Aβ group. The experiments were independently repeated three times

regulate the level of phosphorylation of GSK-3β. Therefore, we further analyzed the level of tau phosphorylation. According to our results, the Y216 phosphorylation level of GSK-3β was downregulated by tanshinone IIA, whereas the S9 phosphorylation level was dramatically upregulated [Figure 4b]. Of note, all these effects of tanshinone IIA were in a dose-dependent manner and were comparable with the positive drug 4-PBA. These results indicated that tanshinone IIA regulated the GSK-3β pathway of Aβ-induced SH-SY5Y cells, leading to a decrease in the phosphorylation level of tau.

DISCUSSION

Neurodegenerative disease is one of the most severe challenges for human beings. To date, the pathogenesis of AD has not been fully understood. Of note, various natural compounds derived from herbs have been found to have excellent biological activity, which could be the potential candidates for the treatment of AD.[5,6,14] Among these natural compounds, tanshinone IIA is an interesting regulatory molecule for AD, which had been reported to attenuate Aβ-induced neurotoxicity.[12,15]

Aβ-induced neurotoxicity is one of the most important pathological features of AD.[1] Alleviating its toxicity is an essential indicator for evaluating drug candidates of AD. In this study, we demonstrate that tanshinone IIA possessed an excellent pharmacological activity to prevent Aβ-induced neurotoxicity of SH-SY5Y cells. In general, this effect is usually generated by inhibiting the apoptotic pathway.[16] By analyzing the typical apoptotic characteristics and the critical factors involved in the apoptotic pathway, our results showed that tanshinone IIA indeed inhibited the Aβ-induced apoptosis of SH-SY5Y cells. These findings indicated that tanshinone IIA had the potential pharmacological activity to become a drug candidate for AD.

Tau protein is a phosphate-containing essential protein in maintaining the stability of microtubule and nerve cell function, which participates in the growth, development, and formation of axons.[17,18] Studies have shown that the level of tau protein phosphorylation in the brain of patients may become an index for early diagnosis of AD.[19] The pathological mechanism of tau phosphorylation has a significant relationship between Aβ peptides. Our findings suggest that tanshinone IIA inhibited the Ser205, Ser396, and Ser413 phosphorylation of tau

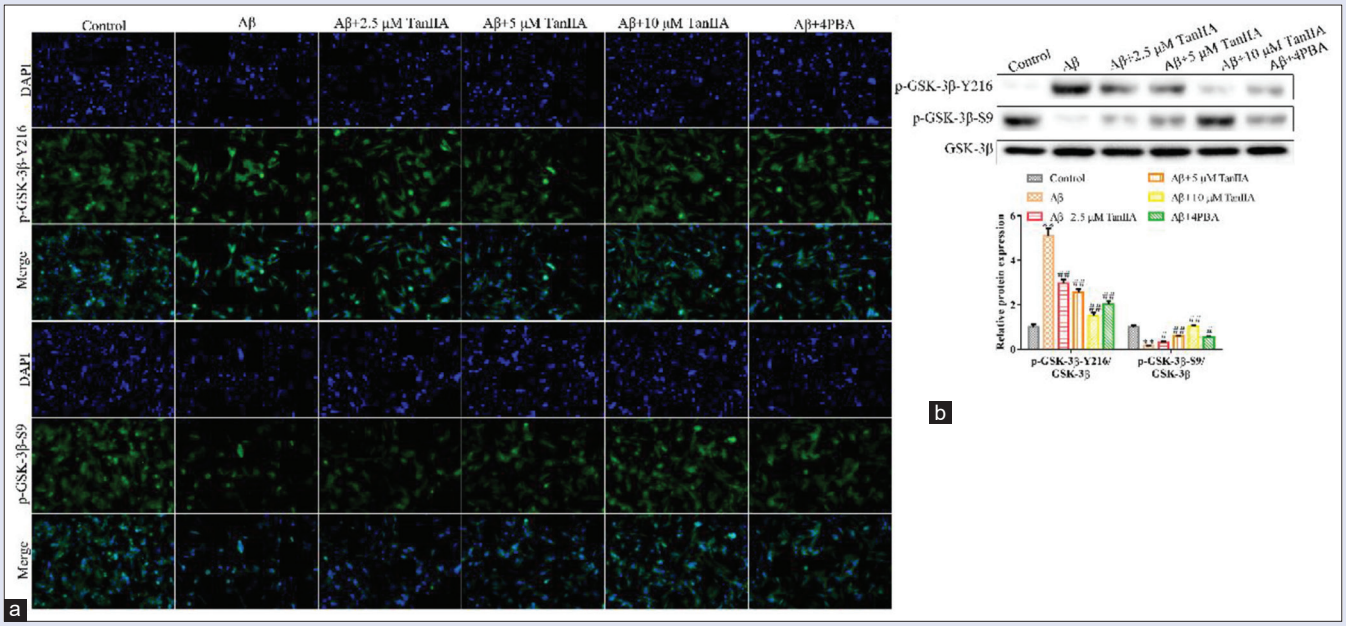


Figure 4: Effects of tanshinone IIA on GSK-3β phosphorylation of SH-SY5Y cells injured by $A\beta_{25-35}$. After treating with sodium 4-phenylbutyrate or indicated concentrations of tanshinone IIA on $A\beta_{25-35}$-injured SH-SY5Y cells, (a) phosphorylation levels of GSK-3β protein were measured by immunofluorescence and (b) protein levels of phosphorylated GSK-3β proteins (Y216 and S9) and total GSK-3β proteins were measured by Western BLOT. Statistical data are presented as mean ± standard deviation $n = 3$, $^{**}P < 0.01$, versus control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus Aβ group. The experiments were independently repeated three times

protein. However, neuroinflammation is another typical pathological feature in the occurrence and development of AD in addition to Aβ deposition and tau hyperphosphorylation.[20,21] Here, we found that the protein and mRNA levels of an inflammatory factor, including TNF-α, IL-6, IL-1β, and IL-10, were downregulated by tanshinone IIA in Aβ-induced SH-SY5Y cells. These findings show that tanshinone IIA decreased the level of phosphorylation of tau, leading to the amelioration of inflammatory response in the Aβ-induced SH-SY5Y cells.

GSK-3β is a critical serine/threonine protein kinase, which plays a crucial role in neuronal development, neurophysiological function, and the pathogenesis of some nervous system diseases.[22] GSK-3β plays a critical role in the production of toxic Aβ protein, abnormal hyperphosphorylation of tau protein, dystrophic neuritis, and impaired neuronal function. Hence, it plays a vital role in pathological transformations. Sengupta *et al.*[23] found that GSK-3β participates in the phosphorylation of multiple sites of tau protein activation. Hoshi *et al.*[24] found that toxic Aβ rapidly increased the phosphorylation of GSK-3β, which contributed to the abnormal hyperphosphorylation of tau protein and neuronal death. In addition, Saeki *et al.*[25] suggested that hyperphosphorylated tau protein activates GSK-3β via oxidative stress, inflammatory response, and apoptosis. However, the roles of tanshinone IIA on the regulation of the GSK-3β pathway of SH-SY5Y cells are not clear. According to our results, the Y216 phosphorylation level of GSK-3β was decreased by tanshinone IIA, whereas the S9 phosphorylation level was increased. These results show that tanshinone IIA regulated the GSK-3β pathway of Aβ-induced SH-SY5Y cells and decreased the phosphorylation level of the tau protein.

CONCLUSION

Our findings demonstrated that tanshinone IIA alleviated neurotoxicity and hyperphosphorylated tau in SH-SY5Y cells induced by $A\beta_{25-35}$ through the GSK-3β pathway, which may provide a novel insight to illustrate the therapeutic mechanism of tanshinone IIA on the AD.

Financial support and sponsorship

This work was supported by the Natural Science Foundation of Anhui Province Education Department (NO. KJ2015A298) and the Quality Engineering Project of Anhui Province Education Department (NO. 2018 mooc438).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol 2018;25:59-70.
2. Morris GP, Clark IA, Vissel B. Questions concerning the role of amyloid-β in the definition, aetiology and diagnosis of Alzheimer's disease. Acta Neuropathol 2018;136:663-89.
3. Gouras GK, Olsson TT, Hansson O. β-Amyloid peptides and amyloid plaques in Alzheimer's disease. Neurotherapeutics 2015;12:3-11.
4. Sengupta U, Nilson AN, Kayed R. The role of amyloid-β oligomers in toxicity, propagation, and immunotherapy. EBioMedicine 2016;6:42-9.
5. Afzal M, Redha A, AlHasan R. Anthocyanins potentially contribute to defense against Alzheimer's disease. Molecules 2019;24:4255.
6. Olasehinde TA, Olaniran AO, Okoh AI. Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Mar Drugs 2019;17:609.
7. Deshpande P, Gogia N, Singh A. Exploring the efficacy of natural products in alleviating Alzheimer's disease. Neural Regen Res 2019;14:1321-9.
8. Li ZM, Xu SW, Liu PQ. *Salvia miltiorrhiza Burge* (Danshen): A golden herbal medicine in cardiovascular therapeutics. Acta Pharmacol Sin 2018;39:802-24.
9. Zhou L, Zuo Z, Chow MS. Danshen: An overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 2005;45:1345-59.
10. Qian YH, Xiao Q, Xu J. The protective effects of tanshinone IIA on β-amyloid protein (1-42)-induced cytotoxicity via activation of the Bcl-xL pathway in neuron. Brain Res Bull 2012;88:354-8.
11. Jiang P, Li C, Xiang Z, Jiao B. Tanshinone IIA reduces the risk of Alzheimer's disease by inhibiting iNOS, MMP-2 and NF-κBp65 transcription and translation in the temporal lobes of rat models of Alzheimer's disease. Mol Med Rep 2014;10:689-94.

12. Geng L, Liu W, Chen Y. Tanshinone IIA attenuates Aβ-induced neurotoxicity by down-regulating COX-2 expression and PGE2 synthesis via inactivation of NF-κB pathway in SH-SY5Y cells. J Biol Res (Thessalon) 2019;26:15.
13. Zhang N, Li WW, Lv CM, Gao YW, Liu XL, Zhao L. miR-16-5p and miR-19b-3p prevent amyloid β-induced injury by targeting BACE1 in SH-SY5Y cells. Neuroreport 2020;31:205-12.
14. Shi LY, Zhang L, Li H, Liu TL, Lai JC, Wu ZB, *et al.* Protective effects of curcumin on acrolein-induced neurotoxicity in HT22 mouse hippocampal cells. Pharmacol Rep 2018;70:1040-6.
15. Yang W, Zhang J, Shi L, Ji S, Yang X, Zhai W, *et al.* Protective effects of tanshinone IIA on SH-SY5Y cells against oAβ 1-42-induced apoptosis due to prevention of endoplasmic reticulum stress. Int J Biochem Cell Biol 2019;107:82-91.
16. Song J, Park KA, Lee WT, Lee JE. Apoptosis signal regulating kinase 1 (ASK1): Potential as a therapeutic target for Alzheimer's disease. Int J Mol Sci 2014;15:2119-29.
17. Lindwall G, Cole RD. Phosphorylation affects the ability of tau protein to promote microtubule assembly. J Biol Chem 1984;259:5301-5.
18. Strömberg K, Eketjäll S, Georgievska B, Tunblad K, Eliason K, Olsson F, *et al.* Combining an amyloid-beta (Aβ) cleaving enzyme inhibitor with a γ-secretase modulator results in an additive reduction of Aβ production. FEBS J 2015;282:65-73.
19. Iqbal K, Liu F, Gong CX. Tau and neurodegenerative disease: The story so far. Nat Rev Neurol 2016;12:15-27.
20. Savarin C, Hinton DR, Valentin-Torres A, Chen Z, Trapp BD, Bergmann CC, *et al.* Astrocyte response to IFN-γ limits IL-6-mediated microglia activation and progressive autoimmune encephalomyelitis. J Neuroinflammation 2015;12:79.
21. Brambilla R. The contribution of astrocytes to the neuroinflammatory response in multiple sclerosis and experimental autoimmune encephalomyelitis. Acta Neuropathol 2019;137:757-83.
22. Hur EM, Zhou FQ. GSK3 signalling in neural development. Nat Rev Neurosci 2010;11:539-51.
23. Sengupta A, Kabat J, Novak M, Wu Q, Grundke-Iqbal I, Iqbal K. Phosphorylation of tau at both Thr 231 and Ser 262 is required for maximal inhibition of its binding to microtubules. Arch Biochem Biophys 1998;357:299-309.
24. Eldar-Finkelman H. Glycogen synthase kinase 3: An emerging therapeutic target. Trends Mol Med 2002;8:126-32.
25. Saeki K, Machida M, Kinoshita Y, Takasawa R, Tanuma S. Glycogen synthase kinase-3β2 has lower phosphorylation activity to tau than glycogen synthase kinase-3β1. Biol Pharm Bull 2011;34:146-9.