Reduction of Aβ Generation by Schisandrin B through Restraining Beta-Secretase 1 Transcription and Translation

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

E 1 **Zeng Li***
B 2 **Xiao-chang Liu***
C 3 **Rui Li**
A 4 **Jun Chang**

1 School of Pharmacy, Anhui Key Laboratory of Bioactivity of Natural Products, Anhui Medical University, Hefei, Anhui, P.R. China
2 Department of Pharmacy, Zhuhai Hospital of Guangdong General Hospital, Zhuhai, Guangdong, P.R. China
3 Department of Pharmacy, Anhui Academy of Medical Sciences, Hefei, Anhui, P.R. China
4 Department of Orthopedics, The Fourth Affiliated Hospital of Anhui Medical University, Hefei, Anhui, P.R. China

Corresponding Author:
Source of support:

* These authors contributed equally to this work
Jun Chang, e-mail: blackpippy@126.com
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Background: Beta-secretase 1 (BACE1) is a rate-limiting enzyme in the generation of amyloid beta peptides, which are associated with Alzheimer's disease (AD). It has been reported that Schisandrin B could improve cognitive functions in animal models of AD, but the underlying mechanisms are not completely understood.

Material/Methods: In this research, in order to investigate the effects of Schisandrin B on amyloid-β (Aβ) metabolism and its mechanisms, amyloid precursor protein (APP) and its proteolytic products were determined by enzyme-linked immunosorbent assay (ELISA), western blotting, and RT-PCR after incubation of N2a/Swe cells with Schisandrin B.

Results: The results indicated that Schisandrin B can significantly reduce the level of secretion of $A\beta_{40}$ and $A\beta_{42}$ secreted in N2a/Swe cells. Additionally, there was nonsignificant change in APP level after Schisandrin B treatment. Treatment of Schisandrin B dramatically reduced the mRNA and protein expression levels of BACE1. Moreover, Schisandrin B treatment resulted in a reduction of protein level of sAPPβ, an APP fragment cleavage by BACE1.

Conclusions: These results suggest that Schisandrin B inhibits the transcription and translation of BACE1, suppresses the activity of BACE1, and ultimately attenuates Aβ generation, which provides a novel mechanism for the regulation of Aβ metabolism by Schisandrin B.

MeSH Keywords: **Alzheimer Disease • Amyloid Precursor Protein Secretases • Neuropharmacology**

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Background

Alzheimer's disease (AD) is a progressive dementia characterized by the deposition of fibrillar β-amyloid (Aβ) in the brain parenchyma and vasculature [1–3]. The pathogenesis of AD is quite complicated. It is unclear which mechanisms lead to the deposition of Aβ. However, as for the pathogenesis of AD to date, "Aβ cascade theory" is generally accepted. In early onset forms of AD, mutations in the genes encoding the β-amyloid precursor protein (APP) or the presenilins (PSs) cause an elevation of total Aβ or the relative increase of longer forms of Aβ, which deposit more readily. This phenomenon further triggers a series of cascade reaction of AD in pathology and physiology [4–6]. Moreover, degeneration and apoptosis of the nerve cell occur, causing dementia [7,8].

Aβ peptides are produced from a membrane-bound APP by sequential proteolytic cleavage by two aspartic proteases, β, and γ-secretase. β-secretase (β-site APP cleaving enzyme, BACE1) has been identified as the enzyme responsible for the initial processing of APP generating the secreted amino-terminal part of APP (sAPPβ) and the membrane-bound carboxy-terminal part C99 [9]. The C99 fragment is subsequently cleaved by γ-secretase, leading to toxic Aβ peptides. It appears that the modulation of BACE1 activity is sufficient to alter Aβ levels in the brain, a process expected to affect Aβ plaque formation [10,11]. Early onset of AD, as well as protection from AD, is associated with genetic alterations in APP, thus implicating that the amyloid pathway and alterations in the production of Aβ are important reasons for the disease. Taken together, this indicates that BACE1 restraining, to stop or reduce the production of Aβ, is an important strategy to treat AD [12,13].

Schisandrin B is a type of lignan extracted from nutlet of Schisandra chinensis (Figure 1). Schisandrin B can resist oxidation and inflammation resistance and decrease cholesterol, which are all closely related to the AD pathogenesis [14,15]. In recent years, researchers have found that Schisandrin B is capable of restraining damage of nerve cell induced by Aβ [16]. This phenomenon indicates that Schisandrin B has potential in curing AD. However, the effect and mechanism have not been identified. In this research, in order to investigate the effects of Schisandrin B on amyloid-β (Aβ) metabolism and its mechanisms, APP and its proteolytic products were determined by enzyme-linked immunosorbent assay (ELISA), western blotting, and RT-PCR after incubation of N2a/Swe cells with Schisandrin B. The results showed that Schisandrin B can decrease β secretase activity by restraining transcription and translation of BACE1. Then, it will affect the generation of Aβ. The present results may provide information about the potential use of Schisandrin B as a new drug to treat AD.
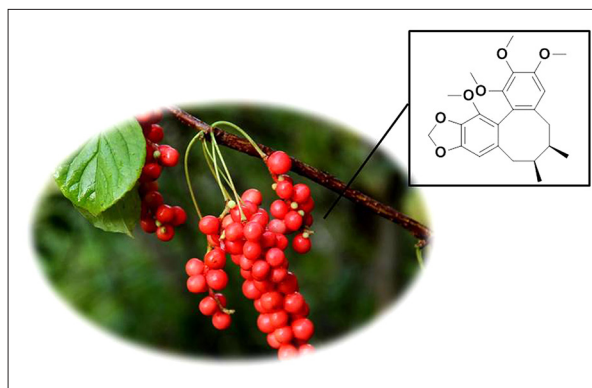
Figure 1. Schisandra sphenanthera and the structure of Schisandrin B.

Material and Methods

Materials

Schisandrin B and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Aladdin Shanghai Biochemical Technology Co., Ltd. (Shanghai, China). Stock solutions of compounds (10 mM) were prepared in DMSO and stored at –80°C. ELISA kits for $A\beta_{40}$ and $A\beta_{42}$ were purchased from Bioval Technologies (Shanghai, China). The two-step RT-PCR kit and the total RNA isolation kit were purchased from SBS Genetech Co., Ltd. (Beijing China). The N2a/Swe cell line was obtained from Bioleaf Co., Ltd. (Shanghai, China). All antibodies were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany).

MTT assay

N2a/Swe cells were seeded in 96-well plates at a concentration of 5×10^3 cells/well and exposed to various concentrations of Schisandrin B (0, 1, 5, 10, and 20 mM). Following treatment for 48 hours, 10 mL MTT solution was added to each well and then incubated for four hours. Following this, 100 mL DMSO was added to each well. Optical density was detected at 490 nm. Three independent experiments were performed.

Detection of $A\beta_{40}$ and $A\beta_{42}$ in culture medium

$A\beta_{40}$ and $A\beta_{42}$ levels were measured using an $A\beta_{40}$ and $A\beta_{42}$ Rapid ELISA Kit according to the manufacturer's instructions.

Western blot

Following four days of treatment with or without Schisandrin B, the N2a/Swe cells were harvested and washed with PBS (pH 7.4) three times. The collected cells were lysed in 150 μL of extraction buffer consisting of 100 μL solution A (50 mM glucose, 25 mM Tris-HCl, pH 8, 0 mM EDTA, and 1 mM phenylmethylsulfonyl

fluoride) and 50 μL solution B (50 mM Tris-HCl, pH 6.8, 6 M urea, 6% 2-mercaptoethanol, 3% sodium dodecyl sulfate, and 0.003% bromophenol blue). The solid-liquoid was centrifuged at 15,000 rpm and 4°C for 5 minutes, and the supernatant (10 μL for each sample) was loaded onto a 10% polyacrylamide gel and transferred onto a microporous polyvinylidenemdifluoride (PVDF) membrane. The experiment was performed using primary antibodies and horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody. Finally, all protein bands were visualized using chemiluminescence substrates.

RT-PCR

After incubation with various concentrations of Schisandrin B, cell pellets were dissolved in TRIzol solution. Total RNA was extracted in accordance with the manufacturer's instructions and mixed with distilled deionized water containing 0.1% diethyl pyrocarbonate (DEPC) to obtain a final volume of 50 μL. Each reaction mixture (20 μL) contained 1 x M-MLV buffer, 100 pmol oligo-dT primer, 100 U of M-MLV reverse transcriptase, 500 μM dNTP, 0.1% DEPC-H_2O, and 1 mg of total RNA. The mixture was incubated at 42°C for 60 minutes for reverse transcription, and at 92°C for 10 minutes to inactivate the enzyme. PCR was carried out through the following steps. Each 20 μL reaction contained 1 x PCR buffer, 1.5 μM c-myc primers, 0.15 μM β-actin primers, 500 μM dNTPs, 0.1% DEPC-H_2O, 1 U of Taq polymerase, and 3 μL of the cDNA template. Each reaction mixture was incubated in a thermal cycler as follows: 95°C for 5 minutes, 36 cycles of 95°C for one minute, 50°C for one minute, and 72°C for one minute. The amplified products were separated in a 1.5% agarose gel, and images were obtained on a Gel Doc 2000 Imager System. The primers used in the real-time RT-PCR were as follows: BACE1A, 5'-TTCCGCATCACCATCCTT-3'; BACE1S, 5'-ATGACCGC TCCCATAACG-3'; β-actinA, 5'-GTTGCTATCCAGGCTGTGC-3'; and β-actinS, 5'-GCATCCTGTCGGCAATGC-3'.

### Statistical analysis

All data were expressed as the mean ± standard deviation unless otherwise specified. Statistical analysis was performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA), followed by Tukey post-hoc tests using GraphPad Prism 5. * $p<0.05$ was considered to indicate a statistically significant difference.

Results

Effect of Schisandrin B on the activity of N2a/Swe cells

The effect of Schisandrin B on N2a/Swe cell activity is detected by MTT assay. The results indicate no significant differences between the activities of cells in each group after the action of
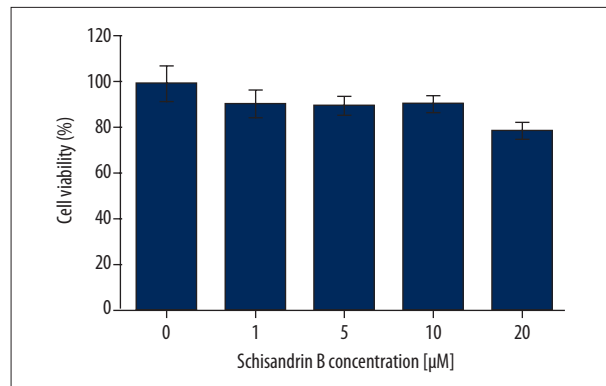
Figure 2. Effect of Schisandrin B on metabolic activity in N2a/Swe cells. Columns with bar represent the means ± standard deviation of the three independent experiments.

Schisandrin B with designed concentration for 24 hours. This finding shows that Schisandrin B exhibits no remarkable toxic and side effects on the growth and living of N2a/Swe within the aforementioned scope of concentration and time (Figure 2).

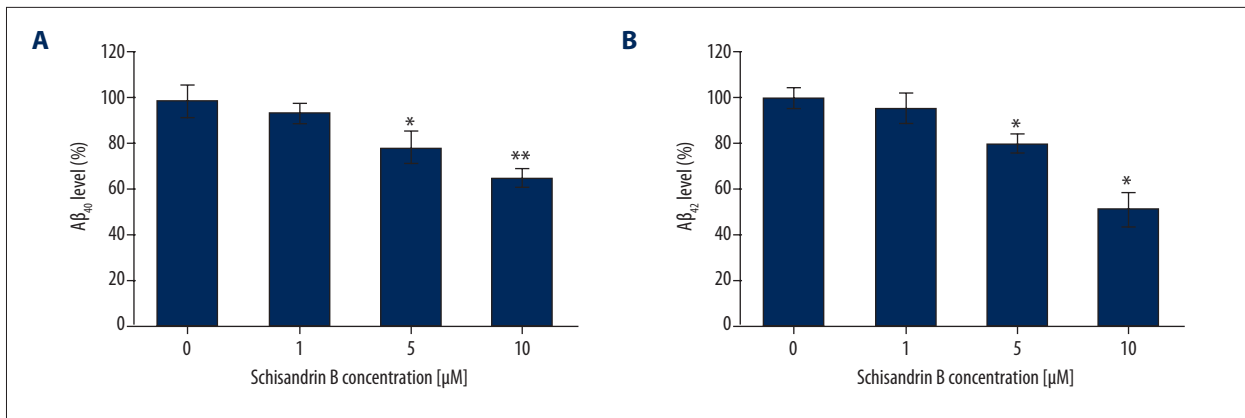### Effect of Schisandrin B on secretion of Aβ$_{40}$ and Aβ$_{42}$ by N2a/Swe cells

To determine whether Schisandrin B affects the metabolism of Aβ, the level of secretion of Aβ40 and Aβ42 by N2a/Swe cell is detected by means of ELISA. The results indicate no significant difference between the exocytosis of Aβ$_{40}$ after the action of Schisandrin B of 1 μM for 24 hours. However, at concentrations of 5 μM and 10 μM, Schisandrin B can noticeably decrease the exocytosis of Aβ$_{40}$. The decrease of Aβ$_{40}$ is concentration dependent (Figure 3A). The detection results of exocytosis of Aβ$_{42}$ also present the same trend (Figure 3B).

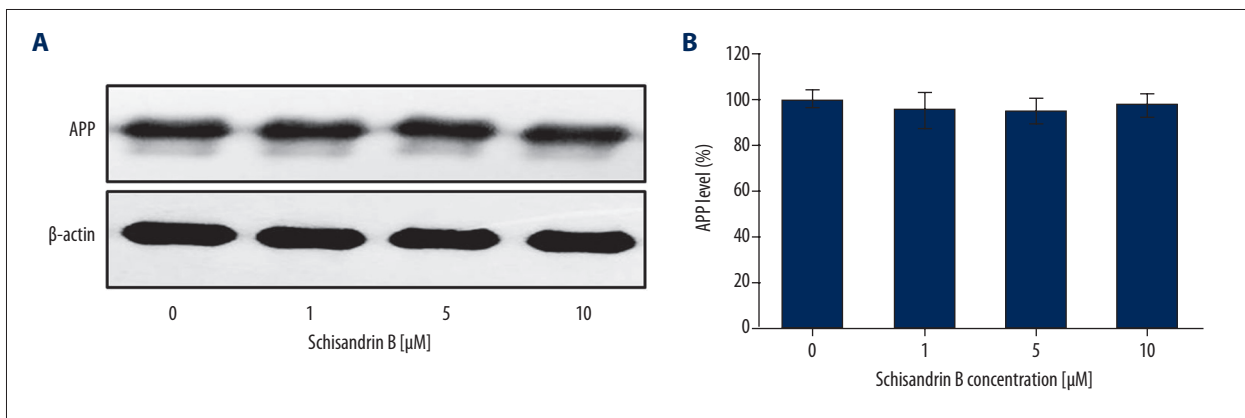### Effect of Schisandrin B on APP protein expression of N2a/Swe cells

Aβ occurs through APP digestion by β secretase and γ secretase. To determine whether the restrain effect of Schisandrin B on Aβ secretion depends on its regulation and control on APP, the expression level of APP of N2a/Swe cell was detected by western blot analysis. The results showed that no significant changes exist on the expression level of APP after the action of Schisandrin B at different concentrations for 24 hours. This finding shows that the reduction of Aβ by Schisandrin B was not obtained by lowering the expression level of APP (Figure 4).

Effect of Schisandrin B on mRNA level and protein expression level of BACE1 of the N2a/Swe cells

First of all, reverse transcription polymerase chain reaction (RT-PCR) method was used to detect the mRNA level of BACE1. The

Figure 3. Effect of Schisandrin B on the supernatant levels of Aβ in N2a/Swe cells. The secreted Aβ levels were measured by ELISA method. (**A**) Quantitative analysis of the supernatant levels of $Aβ_{40}$ in N2a/Swe cells (* $p<0.05$, ** $p<0.01$). (**B**) Quantitative analysis of the supernatant levels of $Aβ_{42}$ in N2a/Swe cells (* $p<0.05$).

Figure 4. Effect of Schisandrin B on APP protein level in N2a/Swe cells. (**A**) Representative immunoblots of APP from N2a/Swe cells. (**B**) Densitometric analysis of APP normalized to β-actin levels. Values are expressed as percentage of control.

results indicate no significant difference between the mRNA levels of BACE1 of N2a/Swe after the action of Schisandrin B of 1 µM for 24 hours. However, at concentrations of 5 µM and 10 µM, Schisandrin B markedly decreased the mRNA level of BACE1, and the decrease of mRNA of BACE1 presents a trend of dependence on concentration (Figure 5A). Meanwhile, the effect of Schisandrin B on the protein expression level of BACE1 was detected as well. The results are presented in Figure 5B, wherein the decreased trend of protein expression of BACE1 was consistent with the drop of mRNA of BACE1.

Effect of Schisandrin B on enzyme-digested products of α/β secretase of N2a/Swe cells

To further determine the effect of Schisandrin B on the enzyme digestion activity of β secretase, the protein level of sAPPα as the enzyme-digested product of α secretase and that of sAPPβ as the enzyme-digested product of BACE1 are tested. APP is the competitive substrate of α secretase and β secretase. Moreover, once the enzyme digestion activity of β secretase

on APP is restrained, that of α secretase will rise. The results present that the exocytosis of sAPPβ of N2a/Swe cell can be remarkably decreased after the action of Schisandrin B for 24 h. The exocytosis of sAPPα of N2a/Swe cell will also rise (Figure 6). This finding revealed that Schisandrin B can improve the enzyme digestion activity of α secretase while restraining the enzymatic activity of BACE1; thus, APP's enzyme-digested product was changed.

Soluble Aβ oligomers of a wide range of sizes appear to be in a complex equilibrium with the 8 nm fibrils of Aβ that are deposited in insoluble amyloid plaques. There has been considerable debate about which Aβ assemblies are most responsible for inducing cytotoxicity.

Discussion

The most typical neuropathological alteration in AD is the deposition of amyloid plaque, with main component of soluble
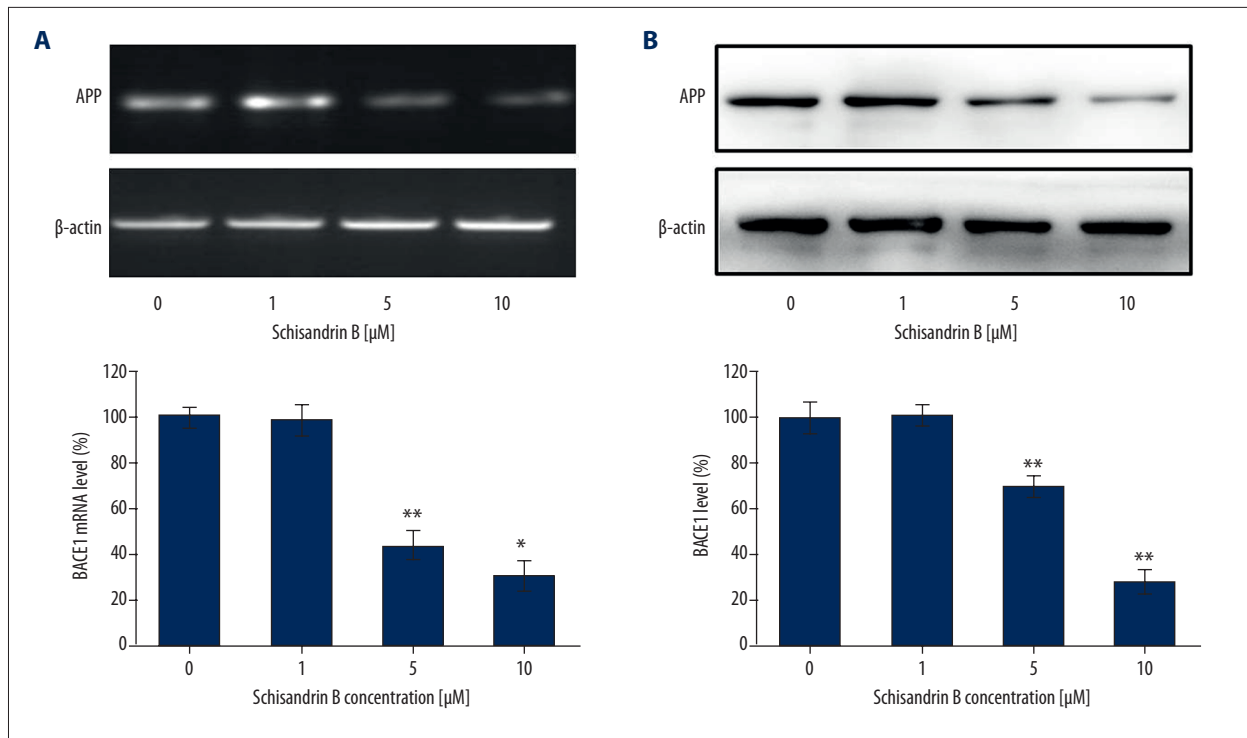
Figure 5. Effect of Schisandrin B on BACE1 mRNA level and protein level in N2a/Swe cells. (**A**) Schisandrin B reduces BACE1 mRNA level in N2a/Swe cells (* $p<0.05$, ** $p<0.01$). (**B**) Schisandrin B reduces BACE1 protein level in N2a/Swe cells (** $p<0.01$).
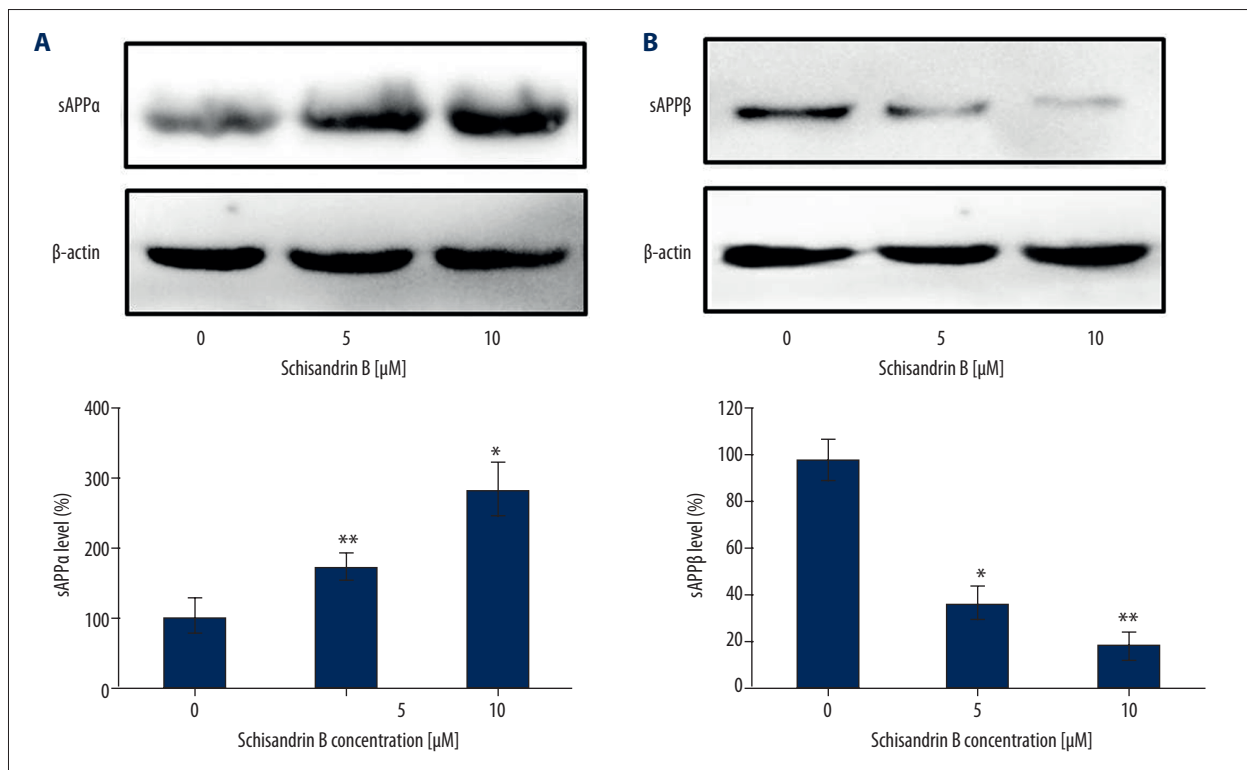
Figure 6. Effect of Schisandrin B on the supernatant levels of sAPPα and sAPPβ in N2a/Swe cells. (**A**) Schisandrin B increases the supernatant level of sAPPα in N2a/Swe cells (* $p<0.05$, ** $p<0.01$). (**B**) Schisandrin B reduces the supernatant level of sAPPβ in N2a/Swe cells (* $p<0.05$, ** $p<0.01$).

Aβ polypeptide fragment. There has been considerable debate about which Aβ assemblies are most responsible for inducing cytotoxicity. Several studies revealed that the excessive aggregation of Aβ is closely related to the cognition impairment of AD [18]. Therefore, Aβ accumulation is identified as the main cause of other pathological alterations of AD, and locates in the core stage of the course of AD.

In this article, the research results first confirmed that Schisandrin B can decrease the generation of Aβ in N2a/Swe cell. Then, to determine whether Schisandrin B reduces the level of Aβ by regulating the expression level of APP, the changes of APP in N2a/Swe cell after the action of Schisandrin B at different concentrations were tested. The results indicated that Schisandrin B at different concentrations had no influence on the expression level of APP. This finding further showed that Schisandrin B regulates the metabolism of Aβ probably through affecting the digestion of APP. Moreover, APP degrades through the path of amyloid peptide and non-amyloid peptide under the action of secretase. The related secretases also respectively included α, β, and γ secretase. β-secretase is a novel membrane-bound aspartic protease and is the key rate-limiting enzyme formed from the toxic Aβ product. Thus, the mRNA and protein expression levels of BACE1 are detected at the same time. These results showed that both the mRNA and protein expression levels of BACE1 of N2a/Swe can be significantly decreased after the action of Schisandrin B for 24 hours. The decrease is also concentration dependent, which indicates that

Schisandrin B restrains the transcription and translation of BACE1. APP is the competitive substrate of α and β secretase. After the activity of β secretase is restrained, the enzyme digestion of APP by α secretase will rise. To further understand the effect of Schisandrin B on APP enzyme digestion, the protein expression level of sAPPα as the enzyme-digested product of α secretase and that of sAPPβ as the enzyme-digested product of BACE1 were respectively tested. The results indicated that the level of sAPPβ as the enzyme-digested product of α secretase can be remarkably decreased and that of sAPPα increases. These results revealed that Schisandrin B can reduce the enzyme digestion activity of β secretase by restraining the transcription and translation of BACE1, affecting the digestion of APP, and then decreasing the generation of Aβ.

Conclusions

The results of this research revealed for the first time that Schisandrin B can restrain the transcription and translation of BACE1 and the enzymatic activity. However, the mechanism by which Schisandrin B affects the transcription of BACE1 and what the target spot is still need further investigations.

Conflicts of interest

None.

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