Effect and mechanism of fuzhisan and donepezil on the sirtuin 1 pathway and amyloid precursor protein metabolism in PC12 cells

PENG GUO1, 3, DESHENG WANG1, XIAOMIN WANG2, HONGLIN FENG1, YING TANG1, RUIHONG SUN1, YAN ZHENG2, LIN DONG2, JIAYING ZHAO1, XIN ZHANG1, SHUYU WANG1 and HONGXU SUN1

1Department of Neurology, The First Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang 150001; 2Beijing Institute for Brain Disorders, Capital Medical University, Beijing 100069; 3Department of Geriatrics, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, P.R. China

Received May 2, 2015; Accepted January 8, 2016

DOI: 10.3892/mmr.2016.4957

Abstract. The present study aimed to determine the effect and mechanism of fuzhisan (FZS) and donepezil on the SIRT1 signaling pathway and the metabolism of the amyloid precursor protein (APP) in PC12 cells. An experimental cell model of PC12 cells with Aβ25-35-induced neurotoxicity was established and cell proliferation was determined by the MTT assay following treatment with donepezil and FZS. In addition, cell apoptosis was determined using DAPI staining and light microscopy. Furthermore, western blot analysis and ELISA were utilized to evaluate the expression levels of associated APP, Aβ40, Aβ42, sAPPα, sAPPβ, ADAM10, sirtuin 1 (SIRT1) and forkhead box O (FoxO) protein. The results indicated that the cell model was successfully established and FZS protected the PC12 cells from the neurotoxic effects of Aβ25-35, in a similar effect to donepezil, in a dose-dependent manner. The expression of APP remained at the same level during the experimental period. The levels of Aβ40, Aβ42 and sAPPβ were downregulated, while as sAPPα, ADAM10, SIRT1 and FoxO expression levels were upregulated. In conclusion, FZS treatment attenuated the Aβ25-35-induced neurotoxicity in vitro. The neuroprotective mechanism of FZS was determined, including the induction of ADAM10 and SIRT1-FoxO pathway, which participated in the process of neuroprotection. The present study identified the neuroprotective function of FZS, which may protect against Aβ-induced toxicity. Therefore, FZS may be used clinically as a beneficial therapeutic drug for the development or progression of Alzheimer’s disease.

Introduction

Alzheimer’s disease (AD) is a degenerative disorder of the nervous system of elderly individuals, and the most common type of dementia (1). AD is predominantly characterized by the progressive loss or decline of cognition and memory function (1). The pathological characteristics of AD include the formation of intracellular neurofibrillary tangles (NFTs) and extracellular neuritic plaques containing amyloid-β (Aβ) peptide (2). The association of NFTs and Aβ is yet to be fully elucidated, however one hypothesis is that Aβ may trigger the hyper-phosphorylation of the tau protein, leading to the impairment of axonal transport and destabilization of microtubules, resulting in neuronal apoptosis (3). Based on the above, it was suggested that the phosphorylation of tau may act as an important process in the pathogenesis of AD.

A previous study identified the dominant mutations in the amyloid precursor protein (APP) gene, which was also discovered in the presenilin 1 gene (PSEN1) and presenilin 2 gene (PSEN2) (4). Aβ1-40 and Aβ1-42 peptides are generated following the sequential cleavage of APP. Aβ1-40 and Aβ1-42 accumulate to form the amyloid plaques, one of the major characteristics of AD (2, 3). Previous studies investigated the role of APP in AD and suggested various hypotheses, however its function remains elusive (5, 6). In addition, the mechanisms of activation of the pathways involved in the process of APP, in normal and AD-ageing remain to be fully clarified. A previous study on APP identified that APP was modulated by phosphorylation and phosphorylation-dependent pathways, directly and indirectly (7). Kojro and Fahrenholz (8) reported that the processing of APP occurs via two alternative pathways, the amyloidogenic and nonamyloidogenic pathways, which serve a role in the activation of β-secretase and α-secretase, respectively.

Sirtuins (SIRTs) or silent information regulators were firstly discovered and extracted in yeast (9). SIRTs are grouped as class III histone deacetylases, that function by removing acetyl groups from lysines through consumption of nicotinamide adenine dinucleotide (NAD) (9). There are seven homologs of SIRTs (1-7) in humans displaying various enzymatic activities and functions (10). SIRT1, 2 and 3 have higher deacetylase activities compared with SIRT4, 5 and 6 (11-14). SIRTs are...
located in different cell components, such as the nucleus (SIRT1, SIRT6 and SIRT7), cytoplasm (SIRT2) and mitochondria (SIRT3, SIRT4 and SIRT5) (15). SIRTs are highly conserved NAD+-dependent enzymes that have beneficial effects on certain age-associated diseases (12,13). Numerous studies investigated the effects of SIRTs on AD in numerous mouse models in vivo and cell models in vitro (16-18), concluding that the SIRT1 overexpression displayed a protective effect on the AD phenotype, with SIRT1 being the only SIRT studied in AD animal or cell models. Thus, a therapeutic strategy for AD was designed based on the SIRT1 activity.

Fuzhisan (FZS) is a Chinese herbal complex prescription, which contains the Scutellaria baicalensis Georgii (Labiatae family), Ginseng root (Araliaceae family), Glycyrrhiza uralensis (Leguminosae family) and Anemone altaica (Araceae family) (19). FZS has been used in the clinical therapy for senile dementia for over fifteen years (19,20). Previous studies indicated that FZS increased the cognitive function of patients with AD or AD animal models (21). In addition, other effects or functions of FZS have been identified, including neurotrophic effects, neuroprotective functions. FZS regulates cell apoptosis, therefore, it may prevent the toxicity in SH-SY5Y neuroblastoma cells resulting from Aβ25-35 accumulation (22). Shirong et al (23) demonstrated that FZS increased the hippocampal acetylcholine levels and enhanced the spatial learning capability. Furthermore, FZS improved glucose metabolism in the brain, and blood flow in the frontal and temporal lobes of patients with AD. However, the specific effects of FZS on tau phosphorylation remain to be identified. In addition, the potential signaling pathways used or the mechanisms for neurotrophic and neuroprotective properties of FZS are elusive.

Therefore, the present study investigated the effects and mechanism of FZS and donepezil on the SIRT1 pathway and APP metabolism in PC12 cells, to identify whether FZS attenuates the Aβ25-35-induced toxicity in the cultured PC12 cells, and the effect underlying the signaling mechanisms.

Materials and methods

Cell culture. The neuronal cell line PC12 was purchased from the Cell Resource Center of Shanghai Institutes, Academy of Sciences (Shanghai, China). The PC12 cells were cultured and grown as a mono-layer of cells in the Roswell Park Memorial Institute 1640 (RPMI-1640) medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 60 µg/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA) and 100 µg/ml streptomycin (Sigma-Aldrich) in 5% CO₂ at 37°C.

FZS preparation. The specimen, extraction methods and the effects of evaluation and analysis of FZS were performed as previously described (19,20). The component of FZS, including the Ginseng root, Anemone altaica, Glycyrrhiza uralensis and Scutellaria baicalensis Georgii, was obtained from the Harbin Pharmaceutical Company (Harbin, China). The four components were mixed in proportions of 2:1:1:1, respectively, and macerated for 40 min in 8 volumes (v/w) of distilled water, and then decocted for 1 h. The filtrate was collected and the residue was decocted for another 1 h with 6 volumes (v/w) of distilled water. The filtrate was pooled and lyophilized (crude extract). Finally, the crude extract of FZS was dissolved in water at a final concentration of 0.5 g/ml (crude drug), and stored at -20°C for further experimental use.

Aβ25-35 peptide preparation. The synthetic Aβ25-35 peptide (purity ≥97%, high-performance liquid chromatography) solution was prepared as previously described (24). Briefly, the Aβ25-35 peptide was dissolved in sterile deionized water at a final concentration of 1.0 mM, and then incubated at 37°C for 3 days to allow for aggregation.

Neurotoxic cell model establishment. The PC12 cell line was cultured in RPMI-1640, supplemented with 10% FBS at 37°C in a humidified atmosphere supplemented with 5% CO₂. RPMI-1640 medium was added to the cells for 3 days, and then replaced with new medium for another 3 days. In order to prepare the experiments, cells were seeded into 24-well plates (2x10⁴ cells/cm²), and after 24 h, Aβ25-35 (10, 20 or 40 µM) was added to the medium. Cells were evaluated and observed under a microscope (CKX-31; Olympus Corporation, Tokyo, Japan) at 24 and 48 h following Aβ25-35 incubation.

Effect of FZS and donepezil on cultured cells. PC12 cells were seeded in 24-well plates and divided into two groups for Aβ25-35 treatment as follows: i) Donepezil group, treatment with 20 mM donepezil; ii) FZS group, treatment with 2.5, 5, 15, 45, 90 or 270 µg/ml FZS. Cells that were not treated with the therapeutic agents were designated as the control group. Following incubation for 24 or 48 h, cells were cultured, harvested and subjected to the different experiments.

Protective effect of FZS and donepezil on cells treated with Aβ25-35. PC12 cells were seeded in 24-well plates and divided into three groups for Aβ25-35 treatment as follows: i) Aβ25-35 injury group, 20 mM Aβ25-35 treatment; ii) donepezil (Sigma-Aldrich) protection group, 20 µM donepezil were added to 1 ml culture medium 2 h prior to Aβ25-35 injury; iii) FZS protection group, 2.5, 5, 15, 45, 90, 135 or 270 µg/ml FZS were added to 1 ml culture medium 2 h prior to Aβ25-35 injury. Following incubation for 24 or 48 h, cells were cultured, harvested and subjected to the different experiments.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. PC12 cells were cultured in 96-well plates and treated with 20 µM Aβ25-35, FZS or donepezil for 24 h. MTT (5 mg/ml; Sigma-Aldrich) was added into each well and incubated at 37°C for 4 h. The MTT reaction was terminated by removing the supernatant and dimethyl sulfoxide (Sigma-Aldrich) was added to dissolve formazan products. Finally, the 24-well plates were assessed at the wavelength of 405 nm on a 550 Bio-Rad microELISA plate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each experiment was repeated a minimum of three times.

Detection of apoptosis. Apoptosis was detected according to the alterations in nuclear morphology. The nuclei were
stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) fluorescent DNA dye (Tiangen Biotech Co., Ltd., Beijing, China). Briefly, PC12 cells were cultured in RPMI-1640 and treated with FZS (0, 5, 15, 45 and 90 µg/ml) for 24 h. Following treatment with FZS and permeabilization, cells were incubated with 2 mg/ml DAPI in methanol at 37°C for 30 min. A fluorescence microscope (IX70; Olympus Corporation) was used to observe cell apoptosis at 300-500 nm UV excitation.

Microscopy. The morphological alterations of the PC12 cell models were observed using a light microscope (Olympus Corporation). During the experimental period, cell morphology was observed and evaluated under the CKX-31 light microscope at various time points.

Enzyme-linked immunosorbent assay (ELISA). The capture antibody, mouse monoclonal anti-human anti-p-Shc (cat. no. sc-81520; 1:1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), was coated at the final concentration of 2 g/ml in the antibody coating buffer solution in 96-well plates at 4°C for 24 h. Following incubation with the antibody, cells were washed 4 times with Tris-buffered saline Tween-20 (TTBS; Tiangen Biotech Co., Ltd.) for 5 min, and then blocked with TTBS starting block buffer (Pierce Biotechnology, Inc., Rockford, IL, USA) at room temperature for 1 h. Aβ40, Aβ42, sAPPα, sAPPβ, SIRT1, forhead box O (FoxO) standards and biotinylated 4G8 reporter antibody, at 0.5 g/ml in 20% Pierce Biotechnology, Inc. SuperBlock; 1:1,000; BioLegend, Inc., San Diego, CA, USA; cat. no. SIG-39240-500) were added to the cells, and incubated at 20°C for 2 h. Cells were then washed with TTBS and incubated with streptavidin-hors eradish peroxidase (Santa Cruz Biotechnology, Inc.) at 20°C for 1 h. Subsequently, the fluorogenic substrate Amplex Ultra Red (Molecular Probes; Thermo Fisher Scientific, Inc.) was added to the cells and incubated in RPMI-1640 for 15 min. The reaction products were quantified and examined using the Tecan Genios Pro plate reader (Tecan Group, Ltd., Männedorf, Switzerland) at the wavelength of 450 nm excitation and 535 nm emission.

Western blotting. The PC12 cells were harvested and lysed with the radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). Lysates were extracted and protein concentration was determined using the Bicinchoninic Acid Assay kit (Bio-Rad Laboratories, Inc.). Proteins were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Following blocking with 5% non-fat milk in phosphate-buffered saline and Tween 20 (PBST; pH 7.6) at 4°C overnight, the membranes were incubated with polyclonal rabbit anti-human SIRT1 (1:1,000; cat. no. sc-15404), polyclonal mouse anti-human APP (1:2,000; cat. no. sc-117075), goat polyclonal anti-human Aβ40 (1:1,000; cat. no. sc-7496), rabbit polyclonal anti-human Aβ42 (1:1,000; sc-134426), monoclonal mouse anti-human against sAPPα and anti-sAPPβ (1:1,000; cat. no. sc-69796), rabbit polyclonal anti-human A disintegrin and metalloproteinase domain-containing protein (ADAM)10 (1:1,000; cat. no. sc-25578) and anti-FoxO polyclonal antibodies (1:1,000; Abcam, Cambridge, MA, USA; cat. no. ab195977). All the antibodies were obtained from Santa Cruz Biotechnology, Inc. and incubated overnight at 4°C. Subsequently, the membranes were incubated with goat anti-rabbit polyclonal antibody (1:1,000; OriGene Technologies, Inc., Beijing, China) or rabbit anti–goat polyclonal antibody (1:1,000; Abcam; cat. no. ab39594) for 1 h at room temperature. Finally, the membranes were stained with enhanced chemiluminescence reagent (EMD Millipore, Billerica, MA, USA). Western blot bands were analyzed with the Quantity One software, version 2.0 (Bio-Rad Laboratories, Inc.) to evaluate protein expression.

Statistical analysis. Quantitative and statistical analysis of immuno-blots bands were performed using GraphPad Prism software, version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Briefly, the blots images were scanned with Typhoon (Pharmacia; GE Healthcare Life Sciences, Uppsala, Sweden), digitalized and saved as a TIF format. The relative protein expression of each blot was determined. Data are presented as the mean ± standard deviation of at least three independent experiments. Statistical analysis was performed using the t-test, and the differences among two groups or more were determined using one-way or two-way analysis of variance, respectively. P<0.05 was considered to indicate a statistically significant difference. All experiments were repeated for a minimum of three times.

Results

Neurotoxic cell model is successfully established following treatment with Aβ25-35. Following Aβ25-35 administration, the cell viability of PC12 cells was evaluated using the MTT assay. The results demonstrated that upon 20 µM Aβ25-35 treatment the cell viability was reduced by ~40% compared with the control group (P<0.01; Fig. 1). The results indicated that the neurotoxic cell model of PC12 cells was successfully established.

FZS and donepezil have a protective effect on PC12 cells. Donepezil (20 mM) and FZS (90 µg/ml) treatments had a protective effect on PC12 cells compared with the control group (P<0.01; Fig. 2). Following an increase in the FZS concentration (135 or 270 µg/ml) a toxic effect was observed compared with the control group (P<0.01; Fig. 2).

Protective effect of FZS and donepezil on PC12 cells treated with Aβ25-35. Upon the establishment of the neurotoxic cell model of PC12 cells, donepezil (20 mM) and FZS (2.5, 5, 15, 45, 90, 135 and 270 µg/ml) were used to evaluate the protective effect against Aβ25-35-induced neurotoxicity. The results indicated that FZS and donepezil had protective effects on cell models (Fig. 3).

FZS inhibits apoptosis in PC12 cells treated with Aβ25-35. PC12 cells were incubated with Aβ25-35 (20 µM) for 24 h and FZS (90 µg/ml), and the anti-apoptotic effect of FZS was evaluated using DAPI staining (Fig. 4A). Compared with the control group, 90 µg/ml FZS treatment protected the cells from apoptosis (P<0.01; Fig. 4B) and resulted in observable morphological alterations. The morphological alterations illustrated typical apoptotic characteristics, including...
neurotoxicity induced by the Aβ25-35 peptide treatment in a concentration-dependent manner. These results were consistent with a previous study which indicated that FZS protected the neuroblastoma cell line SH-SY5Y from the Aβ-induced cell apoptosis (20). In addition, another study indicated that FZS protected cortical neurons from Aβ-induced apoptosis (22).

Upon confirmation of the protective effect of FZS on the cell model, relevant protein expression levels were determined using the western blotting and the ELISA assays. The results indicated that FZS had a role in the APP/Aβ processing in AD. Compared with the control group, the expression levels of APP remained stable, where as Aβ40, Aβ42 and sAPPβ expression levels were downregulated and sAPPα was upregulated.

APP is an important component of the amyloid cascade and AD (4). APP is processed or cleaved by numerous pathways, including α, β or γ cleavage forms (4). Among the above cleavage form, the most important physiological route involving cleavage is the cleavage by α-secretase along with the secretory pathway, which ranges from Golgi to plasma membrane (4). A previous study demonstrated that the α-secretase cleavage mainly appears within the localization of Aβ in APP (4). Furthermore, cleavage of α-secretase leads to the secretion of soluble extracellular APP, named sAPPα (6).

Although numerous cells possess a basal level of α-secretase activity, the proteolysis of APP by the cleavage of α-secretase is increased by diverse intracellular pathways, for example, the activation of the protein kinase C (PKC) (5). The activation or reactivation of other membrane receptors coupled to PKC was indicated to enhance the APP cleavage by α-secretase. Furthermore, the experimental findings illustrated that the sAPPα may have a role in the neuroprotective function in the processes of memory and learning capability (6). Therefore, it was hypothesized that the metabolism of APP or its regulation via α-secretase pathway may be correlated with the mechanism of AD pathogenesis. Hartmann et al (25) demonstrated that the ADAMs are capable of cleaving the APP in different cell systems, at the α-cleavage domains. At present, the most commonly suggested ADAMs for candidate α-secretases include ADAM10, ADAM9 and ADAM17 (25). These ADAMs illustrate the identified structures and are all sensitive to the peptide hydroxamates, however, a previous study demonstrated that ADAM17 does not possess inducible α-secretase activity (25). Another study indicated that ADAM10 has a PKC-stimulated α-secretase activity and other classical characteristics, excluding the APP proteolytic processing (26). Evaluation of the α-secretase activity is critical in patients with AD, and may be significant for acknowledging the role of α-secretase in AD pathogenesis and progression (27).

The current study demonstrated that the levels of ADAM10 were significantly increased in PC12 cells treated with FZS. In addition, ADAM10 was involved in the basal unstimulated APP processing, and may be involved in the progression of AD (28). In the present study, the reduction or increase of ADAM10 were hypothesized to trigger the β-secretase amyloidogenic cleavage of APP. This hypothesis was verified by a previous study, which demonstrated that increased sAPPα release and α-secretase activity subsequent to reduction of cholesterol in neuronal cell lines may lead to the reductive secretion of Aβ and sAPPβ (29).
The precise biochemical mechanism for the sAPPα and Aβ formation remains unclear, although numerous studies have indicated the abnormal formations of these two parameters in patients with AD (30). The production or release of APP from platelets is associated with two critical intracellular signaling pathways, the PKC activation pathway and the cyclooxygenase pathway. Multiple intracellular signaling pathways may have an effect on the reduced thrombin-induced αAPP release in patients with AD (31). Therefore, the current study suggested that the reduced ADAM10 levels and the modified intracellular cascade may regulate the processing and trafficking of APP.

Patel et al (32) identified the protective effect of SIRT1 on AD, and demonstrated that calorie restriction reduced the Aβ levels and plaque formation in transgenic AD mouse brains. In addition, a reduction in Aβ has been demonstrated in the cortex of starved squirrel monkeys, and is inversely correlated

Figure 2. Cell viability of PC12 cells treated with FZS and donepezil. ""P<0.001, vs. the control group. FZS, fuzhisan.

Figure 3. Protective effect of FZS and donepezil on cultured cells treated with Aβ25-35. ""P<0.001, vs. the control group. FZS, fuzhisan; Aβ, amyloid-β.

Figure 4. FZS protected against cell apoptosis and led to the demonstrated morphological alterations. Apoptosis was assessed with 4',6-diamidino-2-phenylinole dihydrochloride. The stained PC12 cells were visualized using a fluorescence microscope. (A) Images of the apoptosis examination. (B) Statistical analysis of the apoptotic rate. ""P<0.001. FZS, fuzhisan.
The above studies suggest that SIRT1 has a neuroprotective effect on AD progression. Furthermore, previous studies demonstrated that SIRT1 activation reduced brain atrophy and neuronal apoptosis induced by the

Figure 5. Morphology of the cell apoptosis under the light microscope (magnification, x400). Upon treatment with FZS for 24 h, the cell morphology indicated plasmic budding, nuclear chromatin condensation and fragmentation and phagocytosis of extruded and apoptotic bodies. FZS, fuzhisan.

Figure 6. FZS prevents Aβ25-35-induced APP/Aβ processing. PC12 cells were treated with FZS (5, 15, 45 or 90 µg/ml) for 24 h. (A) Protein expression levels of APP, Aβ40, Aβ42, sAPPα and sAPPβ. β-actin served as a loading control. (B) Enzyme-linked immunosorbent assay analysis on APP, Aβ40, Aβ42, sAPPα, and sAPPβ in the supernatant of nutrient solution. FZS, fuzhisan; Aβ, amyloid β; APP, amyloid precursor protein; sAPPα, soluble APPα peptide.
progression of AD (33,34). SIRT1 deficiency was associated with the enhanced phosphorylated-tau levels in neurons and the number of NFTs in the AD brain (32,33).

The SIRT1 molecule primarily targets the two AD pathological biomarkers, tau protein and Aβ peptide. The phosphorylated tau degradation reduces the neuronal apoptosis and improves the cognitive function in AD mice. However, the tau breakdown is suppressed upon acetylation of the tau protein by the histone acetyltransferase p300. During the process, SIRT1 deacetylates the acetylated tau, and subsequently decreases the tau levels. Furthermore, SIRT1 inhibition may result in the opposite effect, increasing the tau levels and exacerbating the accumulation of the phosphorylated-tau (35).

Furthermore, previous studies indicated that resveratrol administration and overexpression of SIRT1 may reduce the Aβ levels in vitro and in vivo (9,32-34). The Aβ peptide is generated from APP, a physiological protein, and overexpression of SIRT1 stimulates the α-secretase production in neurons and mice models (32,34). SIRT1 regulation has an effect on activation of the retinoic acid receptor pathway and inhibition of the rho-associated, coiled-coil-containing protein kinase 1 (10). Furthermore, SIRT1 inhibits the NF-κB signaling transduction pathway and reduces the Aβ peptide levels (11). The above observations indicated that SIRT1 may be a protective biomarker of AD progression through multiple pathways and mechanisms, including the degradation of tau protein and the decrease of Aβ peptide levels. In the current study, SIRT1 and FoxO levels demonstrated an increasing tendency, indicating that FZS has a role in the regulation of the SIRT1-FoxO signaling pathway.

SIRT1 is used to determine the association between the aging-associated signaling cascades (9,32). Furthermore, SIRT1 is a selective activator of the FoxO signaling pathway, and acts as a selective inhibitor of the NF-κB signaling pathway (36). SIRT1 increases the FoxO-dependent longevity functions, however, it inhibits the NF-κB-dependent processes of inflammation in aging (9,34). Brunet et al (37) demonstrated that the FoxO/Daf-16 and SIRT/Sir2 longevity genes share certain similar functions in C. elegans and human mammalian system. For example, the interaction between the FoxOs and the SIRT1 enhanced the effects against the oxidative stress and increased the cell-cycle arrest (37). A previous study indicated that the SIRTs affect the FoxO-dependent longevity via another mechanism. For example, SIRT1 increases the efficiency of the nuclear translocation and the trapping of FoxO1, which may enhance the targeted gene-specific transcription (38).

The components of FZS that promote the neuron-protective functions remain to be elucidated (39). Ginseng, an important component of FZS, was demonstrated to alleviate numerous ailments, particularly those in patients associated with increased age and memory deterioration (40). A previous study demonstrated that the ginsenoside Rb1 blocked the Aβ25-35 peptide-induced tau phosphorylation via inhibition of the Cdk5 activity (40). Thus, ginseng may inhibit the Aβ-induced neurotoxicity. Other components of FZS, such as anemone altaica, scutellaria baicalensis and glycyrrhiza uralensis, will need further investigation as they may contribute to the its function.

In conclusion, FZS inhibits the Aβ25-35-induced neurotoxicity. Induction of ADAM10 and SIRT1-FoxO pathway may serve a role in the neuroprotective effects of FZS and its pathogenic mechanism. The results of the present study demonstrated novel insights into the neuroprotective function.
of FZS against Aβ-triggered neurotoxicity. Furthermore, FZS may act as a therapeutic drug for the AD progression and pathology.

References


