Salidroside protects PC12 cells from Aβ$_{1-40}$-induced cytotoxicity by regulating the nicotinamide phosphoribosyltransferase signaling pathway

XUJIAO HUANG, SANLI XING, CHUAN CHEN, ZHIHUA YU and JIULIN CHEN

Geriatrics Laboratory, Shanghai Geriatric Institute of Chinese Medicine, Shanghai University of
Traditional Chinese Medicine, Shanghai 200031, P.R. China

Abstract. Alzheimer's disease (AD) is the most common type of senile dementia, which often develops in elderly or presenile individuals. As one of the pathological features of AD, amyloid β-protein (Aβ) causes energy dysmetabolism, thereby inducing cellular damage and apoptosis. Salidroside is the main active component of the traditional Chinese medicine *Rhodiola*. Previous studies have demonstrated that salidroside exerts a regulatory role in energy metabolism. However, the role and the mechanism of action of salidroside in AD remain unclear. Therefore, the present study used Aβ$_{1-40}$ to induce damage in PC12 cells, thereby establishing a cell model of AD. In addition, salidroside treatment was performed to investigate the protective effect of salidroside and the underlying mechanisms. Aβ$_{1-40}$-induced neuronal toxicity reduced cell viability and caused cellular damage. As a result, the expression level of nicotinamide phosphoribosyltransferase (NAMPT) decreased, the synthesis of nicotinamide adenine dinucleotide (NAD$^+$; an energy metabolism-associated coenzyme) became insufficient, and the NAD$^+$/nicotinamide adenine dinucleotide hydride ratio was reduced. Administration of salidroside alleviated Aβ-induced cell damage and increased the expression level of the key protein NAMPT and the synthesis of NAD$^+$. The results of the present study demonstrate that salidroside exerts a protective effect on Aβ$_{1-40}$-damaged PC12 cells. The underlying mechanism may be associated with the regulation of energy metabolism that relies predominantly on the NAMPT signaling pathway.

Correspondence to: Dr Sanli Xing or Dr Chuan Chen, Geriatrics Laboratory, Shanghai Geriatric Institute of Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 365C South Xiangyang Road, Shanghai 200031, P.R. China
E-mail: xingsanli@163.com
E-mail: article_chenchuan@163.com

Introduction

Alzheimer's disease (AD) is the most common form of dementia that affected an estimated 44 million individuals in 2015. This figure is expected to increase to 135 million by the year 2050 throughout the world (1). The main clinical symptoms of AD include memory impairment, decreased self-care ability, aphasia and visuospatial dysgnosia (2). Neuropathological studies have confirmed that extracellular senile plaque deposition, intracellular accumulation of neurofibrillary tangles and neuronal degeneration loss are the main pathological hallmarks of AD (3,4). Deposition of amyloid β-protein (Aβ) in the brain leads to the formation of senile plaques, which represent a primary pathological characteristic of AD (5). Aβ refers to a class of amyloid precursor protein (APP)-derived polypeptides that are formed under the action of β- and γ-secretase. The common subtypes of Aβ are Aβ$_{1-40}$ and Aβ$_{1-42}$, and each exert neurotoxic effects and induce apoptosis. Therefore, reducing Aβ-induced neuron injury and intervening in AD progression are critical for the treatment of AD.

Studies have shown that energy dysmetabolism contributes significantly to the pathogenesis of a variety of ageing-associated diseases and degenerative diseases of the nervous system, including AD (6). Homeostatic imbalance of energy metabolism develops gradually in the brain with age, which promotes Aβ production. Aβ further induces energy dysmetabolism in the brain, resulting in impaired neuronal synaptic function (7,8). Nicotinamide adenine dinucleotide (NAD$^+$) is an important coenzyme in the *in vivo* redox reaction. It participates in energy synthesis through glycolysis, the tricarboxylic acid cycle and mitochondrial oxidative phosphorylation (9). NAD$^+$ may be synthesized *de novo*, although the majority of NAD is synthesized via the salvage pathway from the conversion of nicotinamide into nicotinamide mononucleotide, which is later converted to NAD$^+$ (10). The NAD$^+$/nicotinamide adenine dinucleotide hydride (NADH) ratio is a fundamental indicator of the cellular redox status (11). Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the NAD$^+$ salvage pathway, and it has been demonstrated that NAMPT participates in energy metabolism and maintains the homeostasis of energy metabolism in the brain by regulating NAD$^+$ synthesis; thus, increased expression levels of NAMPT have

been shown to positively regulate NAD^+ levels (12). Knockout of the NAMPT gene enhances the formation of $A\beta$ plaques in the brain (13). Therefore, recent studies have focused on regulation of NAMPT expression for the purpose of protecting against AD and other neurodegenerative diseases (13-15).

Rhodiola rosea is a perennial herb that belongs to the family Crassulaceae and genus *Rhodiola*. *Rhodiola* is a traditional herbal medicine commonly used across Asia and grows at altitudes of 1,800-2,500 m in cold, pollution-free zones. *Rhodiola* stimulates the nervous system, improves work efficiency, alleviates fatigue and prevents mountain sickness. Salidroside is the primary active ingredient of *Rhodiola*. Various studies have shown that salidroside inhibits the inflammatory response (16), reduces oxidative stress-induced damage (17) and exerts an anticancer effect (18). Certain studies demonstrated that salidroside has a neuroprotective effect, for example, salidroside inhibited $A\beta$-induced neurotoxicity by regulating the phosphatidylinositol 3-kinase/Akt signaling pathway in primary cultured neurons (19). In a rat model of AD, salidroside improved memory impairment of rats (20), and salidroside reduced mitochondrial swelling and attenuated the injury in the central nervous system (21). However, whether the neuroprotective effects of salidroside involve regulation of the $NAMPT/NAD^+$ signaling pathway in PC12 cells remains unknown.

To gain further insights into the neuroprotective functions of salidroside, a PC12 model was used in the present study and the underlying mechanisms were investigated. Whether the neuroprotective effects of salidroside increasing NAD levels against $A\beta_{1-40}$-induced neurotoxicity in PC12 cells were elucidated in the current study. Furthermore, the possible underlying mechanisms were examined by investigating NAMPT expression levels.

Materials and methods

Preparation of $A\beta_{1-40}$. The lyophilized powder of $A\beta_{1-40}$ (cat no. A1075; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was diluted with sterile distilled water to a concentration of 200 μmol/l. Subsequently, $A\beta_{1-40}$ was incubated at 37°C for 7 days and stored at 4°C for further assays.

Cell culture and treatment. Highly differentiated PC12 rat adrenal pheochromocytoma cells (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) were maintained in Dulbecco's modified Eagle's medium (DMEM; cat no. 12100046) containing Gibco 10% fetal bovine serum (cat no. 10099-141) (both from Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 1% penicillin-streptomycin. The cells were maintained in a 37°C incubator in an atmosphere of 5% CO_2 and grew adherently. The culture medium was replaced daily. Upon reaching 80-90% confluence, the cells were trypsinized for 1-2 min. Once the cells became round, 2 ml of culture medium was added to the cells. The cells were then centrifuged, 1,500 x g for 5 min at room temperature, resuspended, and split into culture dishes. The cells were divided into the following main groups: Normal control group, the $A\beta_{1-40}$ group, the $A\beta_{1-40}$ + salidroside (cat no. B20504; Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China) group, and the salidroside group. The normal control group was cultured conventionally in DMEM and did not receive any treatment. The $A\beta_{1-40}$ group was subjected to 24 h of treatment with 5 μmol/l $A\beta_{1-40}$. The $A\beta_{1-40}$ + salidroside group was subjected to simultaneous $A\beta_{1-40}$ (5 μmol/l) and salidroside (100 μmol/l) treatments for 24 h. The salidroside group was treated with 100 μmol/l salidroside for 24 h.

Cell viability assay. PC12 cells were seeded into 96-well plates and cultured at 37°C in DMEM for 24 h. Once the cells attached stably to the culture surface, various concentrations of $A\beta_{1-40}$ (1, 5 or 10 μmol/l) or salidroside (12.5, 25, 50, 100 or 200 μmol/l) were added to the cells. Following a 24-h treatment, each well of cells was overlaid with 10 μl MTT solution (5 mg/ml; cat no. C0009) obtained from Beyotime Institute of Biotechnology (Haimen, China) and incubated at 37°C for 4 h. The supernatant was discarded, and 150 μl dimethyl sulphoxide was added to each well of cells to fully dissolve the crystals. Subsequently, absorbance was measured at a wavelength of 490 nm using a PowerWave XS microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Lactate dehydrogenase (LDH) activity assay. The cells were subjected to different treatments (medium only, 5 μmol/l $A\beta_{1-40}$, 5 μmol/l $A\beta_{1-40}$ + 100 μmol/l salidroside or 100 μmol/l salidroside). Subsequently, the activity of released LDH was examined according to the instructions provided with the LDH Activity Assay kit (cat. no. G1780; Promega Corporation, Madison, WI, USA). Absorbance was measured at a wavelength of 490 nm. The LDH release was expressed as a percentage relative to the positive control group.

$NAD^+/NADH$ analysis. An $NAD^+/NADH$ quantification kit was used to determine NAD^+ and NADH levels (cat no. k337-100; BioVision, Inc., Milpitas, CA, USA) according to the manufacturer's instructions. The cells were washed with pre-cooled phosphate-buffered saline (PBS), homogenized in 400 μl $NAD^+/NADH$ extraction buffer, and then centrifuged for 5 min at 18,000 x g and 4°C. The resulting supernatants were transferred to fresh Eppendorf (EP) tubes and labelled as NADt samples. Subsequently, 200 μl NADt sample was transferred to fresh EP tubes and heated at 60°C for 30 min (all NAD^+ decomposed, leaving only NADH to be analyzed). After cooling on ice, the samples were quickly centrifuged 12,000 x g for 30 sec at 4°C, and the resulting pellets were discarded. The supernatants were collected and labelled as NADH samples for further assays. Standards (0, 2, 4, 6, 8 and 10 μl) and 40 μl samples were loaded into the wells of a microtiter plate, and appropriate volumes of $NAD^+/NADH$ Extraction Buffer were added to bring the volume to 50 μl. Subsequently, 100 μl enzyme reaction mix and 10 μl NADH developer were added to each well. The mixtures were allowed to react at room temperature (RT) for 1-4 h, and the absorbance was measured using a microplate reader (wavelength, 450 nm).

Immunofluorescence assay. Following various treatments and interventions, all groups of cells were washed with PBS for 5 min. The cells were then fixed with 4% paraformaldehyde for 15 min, blocked with a blocking solution containing 10% calf serum for 45 min at RT, incubated with anti-NAMPT antibody (dilution, 1:200; cat no. ab45890; Abcam, Cambridge,

MA, USA) at 37°C for 1 h, and placed in a 4°C refrigerator overnight. After washing with PBS, the cells were incubated with fluorescein isothiocyanate-labelled goat anti-rabbit IgG (dilution, 1:500; cat no. A0562; Beyotime Institute of Biotechnology) for 1 h in a 37°C incubator. After 3 rinses with PBS, the cells were covered with DAPI staining solution (cat no. C1005; Beyotime Institute of Biotechnology) and incubated at RT for 5 min. The cells were rinsed 3 more times with PBS (5 min each time). Subsequently, the cells were mounted onto glass slides in PBS and glycerol (1:1). The fluorescence signal was examined using an Olympus BX51 biological microscope at a magnification of x200.

Western blot analysis. At the end of the treatment period, all groups of cells were collected. Following centrifugation 1,500 x g for 5 min at RT, the cells were lysed in lysis buffer. The resulting supernatants were extracted and the protein concentrations in the supernatants were determined using the bicinchoninic acid (BCA) method. Subsequently, the supernatants were mixed thoroughly with 5X sodium dodecyl sulphate (SDS) loading buffer, boiled at 100°C for 5 min, cooled on ice and then stored at -20°C. Immediately before the experiments, the protein samples were thawed at RT. The protein samples (50 μg each) were heated at 95°C for 5 min, then separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 2% bovine serum albumin for 1 h and then incubated with the primary antibody (anti-NAMPT antibody, dilution, 1:250; cat no. ab45890; Abcam) at 4°C overnight. Subsequently, the membranes were washed with PBS for 45 min, incubated with horseradish peroxidase-labelled secondary antibody (dilution, 1:2,000; cat no. SA00001-2; ProteinTech Group, Inc., Chicago IL, USA) at 37°C for 1 h, and washed again in PBS for 45 min. Protein bands were examined using an ImageQuant LAS 4000 mini chemiluminescence detector (GE Healthcare Life Sciences, Little Chalfont, UK). GAPDH (dilution, 1:1,000, cat no. 5174S; Cell Signaling Technology, Inc., Danvers, MA, USA) served as a loading control.

Statistical analysis. The results were statistically analyzed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All data are expressed as the mean ± standard error. One-way analysis of variance was used to assess multiple groups of data and Student's t-test was used to compare between two groups and P<0.05 was considered to indicate a statistically significant difference.

Results

Impact of Aβ_{1-40} treatment on the viability of PC12 cells and the protective effect of salidroside. PC12 cells were treated with various concentrations of Aβ_{1-40} (1, 5 or 10 μmol/l) for 24 h, which enabled the analysis of changes in PC12 cell viability under the action of different concentrations of Aβ_{1-40}. The results demonstrated that cell viability declined as the Aβ_{1-40} concentration increased. The viability of PC12 cells decreased by ~15% after treatment with 5 μmol/l Aβ_{1-40}. Therefore, a concentration of 5 μmol/l Aβ_{1-40} was selected to establish the cell model of AD (Fig. 1A).
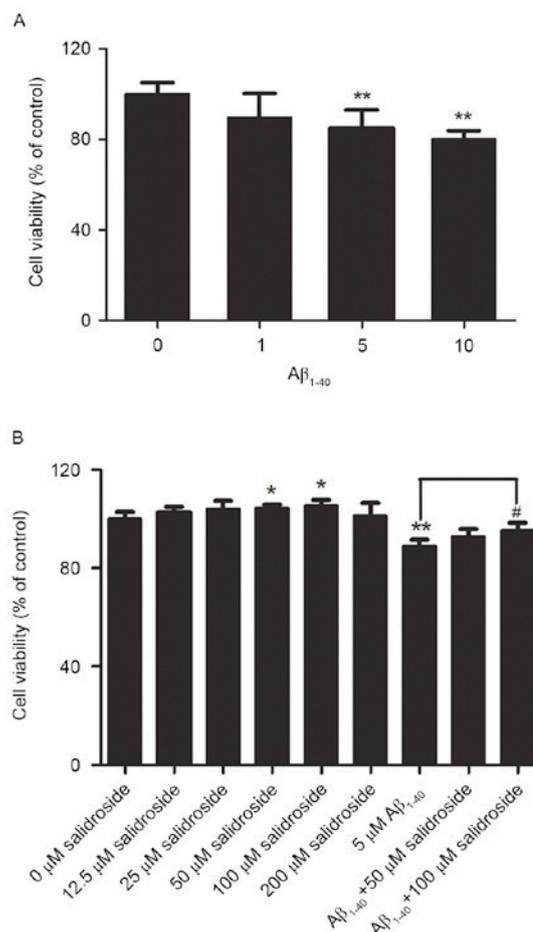

Figure 1. Impact of Aβ_{1-40} treatment on the viability of PC12 cells and the effect of salidroside. (A) Effect of the cytotoxicity of Aβ_{1-40} on PC12 cell viability. PC12 cells were treated with various concentrations of Aβ_{1-40} (1, 5 and 10 μmol/l) for 24 h. (B) Effects of salidroside treatment on the viability of PC12 cells. PC12 cells were treated with various concentrations of salidroside (12.5, 25, 50, 100 and 200 μmol/l) for 24 h, and with different concentrations of salidroside (0, 50, and 100 μmol/l) and exposed to Aβ simultaneously. All data are expressed as the mean ± standard error from four independent experiments. *P<0.05 and **P<0.01 vs. control group; #P<0.05 vs. Aβ_{1-40} group. Aβ, amyloid β-protein.

Before investigation of the protective effect of salidroside, the present study first examined whether salidroside would exert a neurotoxic effect on normal PC12 cells at certain concentrations. PC12 cells were treated with various concentrations of salidroside (12.5, 25, 50, 100 or 200 μmol/l). Compared with the normal control group, 24-h incubation with 12.5, 25 or 200 μmol/l salidroside exerted no significant effect on normal PC12 cells, whereas incubation with 50 and 100 μmol/l salidroside improved cell viability (P<0.05). Therefore, the two concentrations of salidroside (50 and 100 μmol/l) were selected for further investigation of the effect of salidroside on the viability of PC12 cells that were damaged by Aβ_{1-40} exposure. The results indicated that cell viability was significantly decreased in the Aβ_{1-40} group compared with the normal control group (P<0.01). Compared with the Aβ_{1-40} group, treatment with 50 μmol/l salidroside failed to significantly improve the viability of Aβ_{1-40}-damaged cells, whereas treatment with 100 μmol/l salidroside improved the viability of cells damaged by Aβ_{1-40} (P<0.05). The results demonstrated
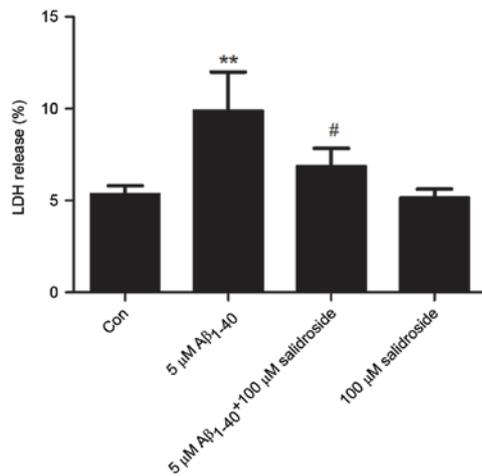
Figure 2. Effect of salidroside treatment on the LDH level of Aβ$_{1-40}$-treated cells. PC12 cells were subjected to different treatments (medium only, 5 μmol/l Aβ$_{1-40}$, 5 μmol/l Aβ$_{1-40}$ + 100 μmol/l salidroside, and 100 μmol/l salidroside) for 24 h. Subsequently, the cells were examined using an LDH Activity Assay kit. All data are presented as the mean ± standard error from four independent experiments (n=4). **P<0.01 vs. control group. #P<0.05 vs. Aβ$_{1-40}$ group. LDH, lactate dehydrogenase; Aβ, amyloid β-protein; Con, control.

that 100 μmol/l salidroside exerted an effect on cell viability and alleviated the Aβ-induced PC12 cell injury. Therefore, this

salidroside concentration (100 μmol/l) was selected for further experiments (Fig. 1B).

Salidroside reduces the LDH level of Aβ$_{1-40}$-treated cells. The LDH level of a culture supernatant indirectly reflects the effects of Aβ$_{1-40}$ and salidroside on cells. As shown in Fig. 2, the Aβ$_{1-40}$ group displayed a significantly increased LDH level compared with that of the normal control group (P<0.01). By contrast, the LDH level was markedly reduced in the Aβ$_{1-40}$ + salidroside group compared with the Aβ$_{1-40}$ group (P<0.05). The results demonstrated that salidroside reduced the Aβ$_{1-40}$-induced cellular damage.

Salidroside increases the NAD$^+$ level and NAD$^+$/NADH ratio of Aβ$_{1-40}$-treated cells. NAD$^+$ and NADH are coenzymes that are necessary for cellular energy metabolism. Under normal physiological conditions, the NAD$^+$/NADH ratio reflects the cellular redox status and is in a constant state of dynamic equilibrium. Disorders of cellular energy metabolism are characterized by changes in NAD$^+$ and NADH levels, and an imbalance in the NAD$^+$/NADH ratio (22). In the present study, the Aβ$_{1-40}$ group exhibited a decreased level of NAD$^+$ compared with that of the normal control group (P<0.01; Fig. 3A). However, the NADH level did not change significantly in the Aβ$_{1-40}$ group compared with the normal control group (Fig. 3B). As a result, the NAD$^+$/NADH ratio was reduced in the Aβ$_{1-40}$
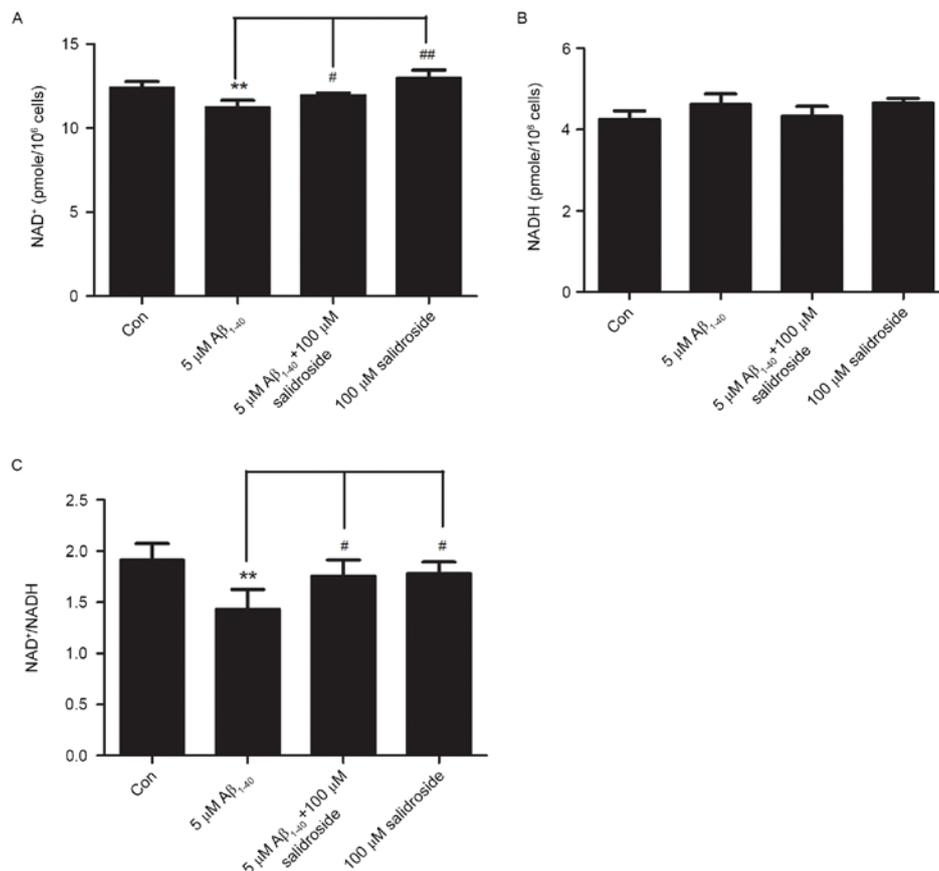

Figure 3. Effect of salidroside treatment on the NAD$^+$ level and NAD$^+$/NADH ratio of Aβ$_{1-40}$-treated PC12 cells. (A) Effect of salidroside treatment on the NAD$^+$ level in the PC12 cells damaged by Aβ$_{1-40}$ exposure. (B) Effect of salidroside treatment on the NADH level in the PC12 cells. (C) Effect of salidroside treatment on the NAD$^+$/NADH ratio in Aβ$_{1-40}$-induced damaged PC12 cells. All data are expressed as the mean ± standard error from four independent experiments (n=4). **P<0.01 vs. control group. #P<0.05 and ##P<0.01 vs. Aβ$_{1-40}$ group. NAD$^+$, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide hydride; Aβ, amyloid β-protein; Con, control.
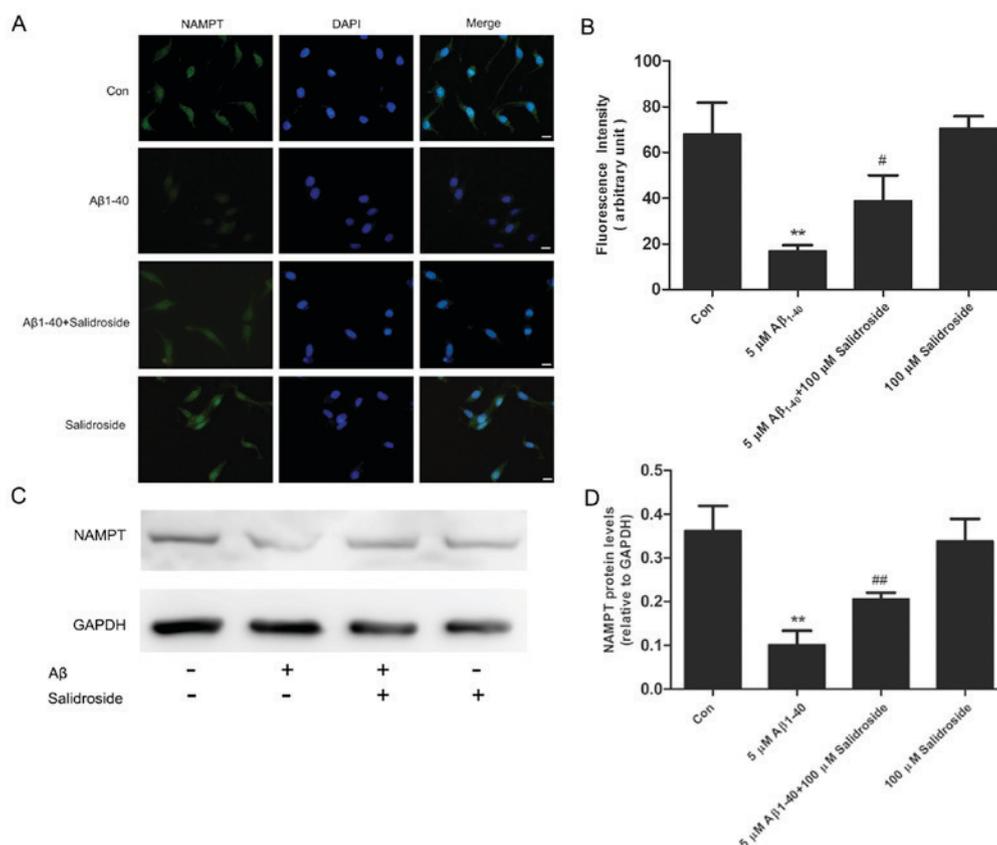
Figure 4. Salidroside upregulates the expression level of NAMPT. (A) NAMPT were detected by immunofluorescence staining and a representative photomicrograph is presented (scale bar, 10 μm). (B) The fluorescence intensity of the NAMPT protein. All data are expressed as the mean ± standard error from three independent experiments. **P<0.01 vs. control group. #P<0.05 vs. Aβ_{1-40} group. (C) Western blot analyses of PC12 lysates from the control (without any treatment), Aβ_{1-40} (with 5 μmol/l Aβ_{1-40} treatment), Aβ_{1-40} + salidroside (5 μmol/l Aβ_{1-40} and 100 μmol/l salidroside simultaneously) and salidroside (100 μmol/l salidroside only) groups. (D) Quantitation of NAMPT protein expression levels. Data are presented as the mean ± standard error of three independent experiments. **P<0.01 vs. control group. ##P<0.01 vs. Aβ_{1-40} group. NAMPT, nicotinamide phosphoribosyltransferase; Aβ, amyloid β-protein; Con, control.

group (P<0.01; Fig. 3C). No significant difference was identified in the NADH level between the Aβ_{1-40} + salidroside group and the Aβ_{1-40} group (Fig. 3B). By contrast, the Aβ_{1-40} + salidroside group demonstrated a significant increase in the NAD⁺ level (P<0.05; Fig. 3A) and the NAD⁺/NADH ratio (P<0.05; Fig. 3C).

Salidroside upregulates the expression level of NAMPT. NAMPT is the rate-limiting enzyme in the salvage pathway of NAD⁺ biosynthesis and is important in regulating the homeostasis of energy metabolism. The results of an immunofluorescence assay demonstrated that the NAMPT-derived fluorescence signal was reduced following 24 h of Aβ_{1-40} treatment, whereas salidroside treatment increased the NAMPT fluorescence signal (Fig. 4A and B). In addition, western blot analysis demonstrated that NAMPT expression was decreased in the Aβ_{1-40} group compared with the normal control group. Salidroside treatment significantly increased the expression level of NAMPT (Fig. 4C and D). Thus, the results indicate that salidroside regulates NAMPT protein expression.

Discussion

AD is the most common cause of dementia in the elderly, but the exact pathogenetic mechanisms underlying AD remain

uncertain. In previous studies, one extensively investigated mechanism is Aβ-mediated neurotoxicity; the aggregates of Aβ peptides exhibit cytotoxicity *in vivo* and *in vitro* (23). Aβ_{1-40} and Aβ_{1-42} are two major forms of Aβ, although Aβ_{1-42} is markedly more prone to aggregation and more toxic to neurons than Aβ_{1-40}; however, Aβ_{1-40} was the predominant isoform of the most abundant cleaved form of APP (~90%) and exerts a toxic effect on neurons in the AD brain (24). In addition, the deposition of Aβ_{1-40} is required for the development of mature amyloid plaques from the initial deposition of Aβ_{1-42}, which was regarded as an early pathological process of AD, and Aβ_{1-40} is often used to establish the model of AD *in vivo* and *in vitro* (25). Injecting 5 mg/ml Aβ_{1-40} caused hippocampal neuronal loss or functional impairment and is thus considered as an indication of energy metabolism (26). As Aβ_{1-40} possesses neuronal toxicity and is capable of inducing neuronal neurotoxicity, PC12 cells treated with Aβ_{1-40} were used as the cell model of AD in the current study. The neuronal toxicity of Aβ_{1-40} is reflected in its ability to cause energy dysmetabolism, and induce cell damage and death (27,28). A number of previous studies used PC12 cells to investigate the molecular mechanisms underlying the development of degenerative diseases of the nervous system (29-32). Therefore, the present study employed the PC12 cells that were damaged by Aβ_{1-40} exposure as the cell model of AD and the significance of NAMPT in AD was evaluated.

The present study demonstrated that Aβ_{1-40} treatment reduced the NAD$^+$ expression level. The underlying reason may be associated with decreased NAMPT expression. As the rate-limiting enzyme in the NAD$^+$ salvage pathway, NAMPT promotes the production of NAD$^+$ (33). Previous studies demonstrated that an elevated NAMPT level reduces cell death (34,35), which is consistent with the results of the present study, which indicate that salidroside increased NAMPT expression and improved cell survival. In addition, the present study identified that salidroside significantly reduced the level of LDH released when compared with that in the Aβ_{1-40} group. The results of the LDH assay were consistent with those of the MTT assay, demonstrating that salidroside treatment attenuated the Aβ_{1-40}-induced PC12 cell injury and exerted a protective effect on PC12 cells.

The expression level of Aβ increases as AD progresses, which causes mitochondrial dysfunction and energy metabolism disorders. The elevated Aβ expression level represents one of the reasons behind neuronal damage and apoptosis (36). NAD$^+$ participates in energy metabolism. Furthermore, loss of NAD$^+$ homeostasis leads to decreased sirtuin-1 (SIRT1) activity. Consequently, SIRT1-mediated deacetylation of signaling molecules (including transcription factors and enzymes) is reduced (37,38). Peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) is a coactivator of the nuclear hormone receptor peroxisome proliferator-activated receptor γ, which also undergoes SIRT1-mediated deacetylation and participates in mitochondrial biosynthesis and energy homeostasis (39). The decreased SIRT1 activity in the brain affects the function of the downstream protein PGC-1α in energy synthesis, resulting in a brain energy crisis (40). As NAMPT is important in NAD$^+$ synthesis, the present study investigated whether salidroside exerts its regulatory effect on energy metabolism pathways via NAMPT. Namely, salidroside attenuates the neuronal toxicity of Aβ by further regulating NAD$^+$/NADH levels. Studies have demonstrated that the expression level of NAMPT decreases gradually with age, with the decrease in NAMPT level particularly evident in the brain of AD mice compared with aged healthy mice. Such a decrease exacerbates neuronal apoptosis (34,41,42). By contrast, increased NAMPT expression levels in the hippocampus and cortex improve cognitive function (35). Therefore, novel treatment strategies for AD may involve the administration of salidroside to regulate NAMPT expression and reduce the severity of AD.

The present study demonstrated that salidroside exerted a protective effect on PC12 cells damaged by Aβ_{1-40} exposure. An AD model was established by Aβ_{1-40} treatment. The viability of PC12 cells decreased and the level of LDH release significantly increased. In addition, the protein expression of NAMPT, the level of NAD$^+$ and the ratio of NAD$^+$/NADH were decreased. The above-mentioned effects of Aβ_{1-40} were attenuated following salidroside treatment. In addition, the present study demonstrated that the decrease in NAMPT/NAD$^+$ levels may represent one of the molecular pathological mechanisms of AD.

In conclusion, the present study demonstrated that salidroside exerted a protective effect on PC12 damaged by Aβ_{1-40} treatment. The protective effect of salidroside may reside in its regulatory effect in energy metabolism, such as the increases in NAMPT expression levels and NAD$^+$ production. Therefore,

NAMPT and NAD$^+$ are considered to be key factors in AD, as well as other neurodegenerative diseases. Thus, regulation of NAMPT and NAD$^+$ may serve as novel therapeutic strategies for the treatment of AD.

Acknowledgements

References

1. Van Cauwenberghe C, Van Broeckhoven C and Sleegers K: The genetic landscape of Alzheimer disease: Clinical implications and perspectives. Genet Med 18: 421-430, 2016.
2. Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S and Van der Flier WM: Alzheimer's disease. Lancet 388: 505-517, 2016.
3. Perl DP: Neuropathology of Alzheimer's disease. Mt Sinai J Med 77: 32-42, 2010.
4. Serrano-Pozo A, Frosch MP, Masliah E and Hyman BT: Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med 1: a006189, 2011.
5. Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M and Mahmoudi J: Amyloid-beta: A crucial factor in Alzheimer's disease. Med Princ Pract 24: 1-10, 2015.
6. Yin F, Boveris A and Cadenas E: Mitochondrial energy metabolism and redox signaling in brain aging and neurodegeneration. Antioxid Redox Signal 20: 353-371, 2014.
7. Wang R, Li JJ, Diao S, Kwak YD, Liu L, Zhi L, Büeler H, Bhat NR, Williams RW, Park EA and Liao FF: Metabolic stress modulates Alzheimer's β-secretase gene transcription via SIRT1-PPARγ-PGC-1 in neurons. Cell Metab 17: 685-694, 2013.
8. Wu MF, Yin JH, Hwang CS, Tang CM and Yang DI: NAD attenuates oxidative DNA damages induced by amyloid beta-peptide in primary rat cortical neurons. Free Radic Res 48: 794-805, 2014.
9. Ussher JR, Jaswal JS and Lopaschuk GD: Pyridine nucleotide regulation of cardiac intermediary metabolism. Circ Res 111: 628-641, 2012.
10. Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, Leo O and Andris F: Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol 32: 3225-3234, 2002.
11. Ying W: NAD$^+$/NADH and NADP$^+$/NADPH in cellular functions and cell death: Regulation and biological consequences. Antioxid Redox Signal 10: 179-206, 2008.
12. Revollo JR, Grimm AA and Imai S: The regulation of nicotinamide adenine dinucleotide biosynthesis by Nampt/PBEF/visfatin in mammals. Curr Opin Gastroenterol 23: 164-170, 2007.
13. Villela D, Schlesinger D, Suemoto CK, Grinberg LT and Rosenberg C: A microdeletion in Alzheimer's disease disrupts NAMPT gene. J Genet 93: 535-537, 2014.
14. Imai S and Guarente L: NAD$^+$ and sirtuins in aging and disease. Trends Cell Biol 24: 464-471, 2014.
15. Herrera-Arozamena C, Martí-Marí O, Estrada M, de la Fuente Revenga M and Rodríguez-Franco MI: Recent advances in neurogenic small molecules as innovative treatments for neurodegenerative diseases. Molecules 21: pii: E1165, 2016.
16. Qi Z, Qi S, Ling L, Lv J and Feng Z: Salidroside attenuates inflammatory response via suppressing JAK2-STAT3 pathway activation and preventing STAT3 transfer into nucleus. Int Immunopharmacol 35: 265-271, 2016.
17. Yang ZR, Wang HF, Zuo TC, Guan LL and Dai N: Salidroside alleviates oxidative stress in the liver with non-alcoholic steatohepatitis in rats. BMC Pharmacol Toxicol 17: 16, 2016.
18. Sun KX, Xia HW and Xia RL: Anticancer effect of salidroside on colon cancer through inhibiting JAK2/STAT3 signaling pathway. Int J Clin Exp Pathol 8: 615-621, 2015.
19. Zhang B, Wang Y, Li H, Xiong R, Zhao Z, Chu X, Li Q, Sun S and Chen S: Neuroprotective effects of salidroside through PI3K/Akt pathway activation in Alzheimer's disease models. Drug Des Devel Ther 10: 1335-1343, 2016.

20. Gao J, Zhou R, You X, Luo F, He H, Chang X, Zhu L, Ding X and Yan T: Salidroside suppresses inflammation in a D-galactose-induced rat model of Alzheimer's disease via SIRT1/NF-κB pathway. Metab Brain Dis 31: 771-778, 2016.
21. Zhang W, Peng M, Yang Y, Xiao Z, Song B and Lin Z: Protective effects of salidroside on mitochondrial functions against exertional heat stroke-induced organ damage in the rat. Evid Based Complement Alternat Med 2015: 504567, 2015.
22. Yang Y and Sauve AA: NAD+ metabolism: Bioenergetics, signaling and manipulation for therapy. Biochim Biophys Acta 1864: 1787-1800, 2016.
23. Selkoe DJ and Schenk D: Alzheimer's disease: Molecular understanding predicts amyloid-based therapeutics. Annu Rev Pharmacol Toxicol 43: 545-584, 2003.
24. Cheng YF, Wang C, Lin HB, Li YF, Huang Y, Xu JP and Zhang HT: Inhibition of phosphodiesterase-4 reverses memory deficits produced by $A\beta_{25-35}$ or $A\beta_{1-40}$ peptide in rats. Psychopharmacology (Berl) 212: 181-191, 2010.
25. Miguel-Hidalgo JJ and Cacabelos R: Beta-amyloid(1-40)-induced neurodegeneration in the rat hippocampal neurons of the CA1 subfield. Acta Neuropathol 95: 455-465, 1998.
26. Yue T, Shanbin G, Ling M, Yuan W, Ying X and Ping Z: Sevoflurane aggregates cognitive dysfunction and hippocampal oxidative stress induced by β-amyloid in rats. Life Sci 143: 194-201, 2015.
27. He F, Cao YP, Che FY, Yang LH, Xiao SH and Liu J: Inhibitory effects of edaravone in β-amyloid-induced neurotoxicity in rats. Biomed Res Int 2014: 370368, 2014.
28. Carrillo-Mora P, Luna R and Colín-Barenque L: Amyloid beta: Multiple mechanisms of toxicity and only some protective effects? Oxid Med Cell Longev 2014: 795375, 2014.
29. Han XH, Cheng MN, Chen L, Fang H, Wang LJ, Li XT and Qu ZQ: 7,8-Dihydroxyflavone protects PC12 cells against 6-hydroxydopamine-induced cell death through modulating PI3K/Akt and JNK pathways. Neurosci Lett 581: 85-88, 2014.
30. Guo M, Li D, Shen H, Jin B, Ren Y, Li M and Xing Y: Leptin-Sensitive JAK2 activation in the regulation of Tau phosphorylation in PC12 cells. Neurosignals 24: 88-94, 2016.
31. Chen CL, Tsai WH, Chen CJ and Pan TM: Centella asiatica extract protects against amyloid β1-40-induced neurotoxicity in neuronal cells by activating the antioxidative defence system. J Tradit Complement Med 6: 362-369, 2015.
32. Qian MC, Liu J, Yao JS, Wang WM, Yang JH, Wei LL, Shen YD and Chen W: Caspase-8 mediates amyloid-β-induced apoptosis in differentiated PC12 cells. J Mol Neurosci 56: 491-499, 2015.
33. Jieyu H, Chao T, Mengjun L, Shalong W, Xiaomei G, Jianfeng L and Zhihong L: Nampt/Visfatin/PBEF: A functionally multi-faceted protein with a pivotal role in malignant tumors. Curr Pharm Des 18: 6123-6132, 2012.
34. Ghosh D, Levault KR and Brewer GJ: Relative importance of redox buffers GSH and NAD(P)H in age-related neurodegeneration and Alzheimer disease-like mouse neurons. Aging Cell 13: 631-640, 2014.
35. Stein LR, Wozniak DF, Dearborn JT, Kubota S, Apte RS, Izumi Y, Zorumski CF and Imai S: Expression of Nampt in hippocampal and cortical excitatory neurons is critical for cognitive function. J Neurosci 34: 5800-5815, 2014.
36. Cabezas-Opazo FA, Vergara-Pulgar K, Pérez MJ, Jara C, Osorio-Fuentealba C and Quintanilla RA: Mitochondrial dysfunction contributes to the pathogenesis of Alzheimer's disease. Oxid Med Cell Longev 2015: 509654, 2015.
37. Zhu XH, Lu M, Lee BY, Ugurbil K and Chen W: In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. Proc Natl Acad Sci USA 112: 2876-2881, 2015.
38. Wang Y: Molecular links between caloric restriction and Sir2/SIRT1 activation. Diabetes Metab J 38: 321-329, 2014.
39. Rodgers JT, Lerin C, Gerhart-Hines Z and Puigserver P: Metabolic adaptations through the PGC-1 alpha and SIRT1 pathways. FEBS Lett 582: 46-53, 2008.
40. Katsouri L, Parr C, Bogdanovic N, Willem M and Sastre M: PPARγ co-activator-1α (PGC-1α) reduces amyloid-β generation through a PPARγ-dependent mechanism. J Alzheimers Dis 25: 151-162, 2011.
41. Yoshino J, Mills KF, Yoon MJ and Imai S: Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 14: 528-536, 2011.
42. Ghosh D, LeVault KR, Barnett AJ and Brewer GJ: A reversible early oxidized redox state that precedes macromolecular ROS damage in aging nontransgenic and 3xTg-AD mouse neurons. J Neurosci 32: 5821-5832, 2012.