

Berberine alleviates amyloid β_{25-35} -induced inflammatory response in human neuroblastoma cells by inhibiting proinflammatory factors

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Abstract. The present study investigated the effect of berberine (BBR) on amyloid β_{25-35} ($A\beta_{25-35}$)-induced inflammatory response in human neuroblastoma cells. To model the inflammatory response observed in the central nervous system of patients with Alzheimer's disease, SH-SY5Y and SK-N-SH neuroblastoma cells were induced by $A\beta_{25-35}$ ($5 \mu\text{M}$) for 24 h. Subsequently, cells were treated with BBR or indomethacin for 2 h. The cell survival rate was determined by the MTT assay. The activity of lactate dehydrogenase (LDH) in the cell culture medium was examined by spectrophotometry. The expression levels of inflammatory factors prostaglandin E2 (PGE2) and tumor necrosis factor- α (TNF- α) were determined by ELISA assays. The mRNA and protein expression levels of interleukin (IL)-1 β , cyclooxygenase 2 (COX-2) and tumor necrosis factor receptor 1 (TNFR1) were measured by reverse-transcription-quantitative polymerase chain reaction and Western blotting, respectively. The results indicated that, treatment with $A\beta_{25-35}$ increased the expression levels of PGE2 and TNF- α , increased the activity of LDH, and up-regulated the mRNA and protein expression of COX-2, IL-1 β , and TNFR1. Treatment with BBR down-regulated the expression levels of PGE2 and TNF- α , decreased the activity of LDH, and downregulated the mRNA and protein expression of COX-2, IL-1 β , and TNFR1. Taken together, the present results suggested that BBR suppressed the inflammatory response induced by $A\beta_{25-35}$ in neuroblastoma cells. The mechanism of action may be associated with the inhibition of proinflammatory factors.

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease associated with aging (1). The pathogenic factors of AD are complex. In addition to cholinergic dysfunction, β -amyloid deposition resulting in inflammatory response is one of the most important pathogenic mechanisms of AD (1). Treatment of AD using acetylcholinesterase inhibitors (AChEIs), which inhibit the acetylcholine degradation in synapses, achieved limited results (1). However, epidemiological data suggested that non-steroid anti-inflammatory drugs (NSAIDs), which inhibit both cyclooxygenase 2 (COX-2) and interleukin (IL)-1 β , may reduce the incidence of AD (2). Therefore, anti-inflammatory drugs that reduce the secretion of inflammatory factors in the peripheral or central nervous system have become the focus of research for the treatment of AD (3).

Berberine (BBR) is the active ingredient of the extract from *Coptis chinensis*. It exhibits a variety of biological activities, including anti-inflammatory, antidiabetic, anticancer and anti-arrhythmic effects, prevention of intestinal bacterial infection, dilation of coronary blood vessels, and lowering blood lipids (4). A previous study indicated that BBR can effectively inhibit expression of inflammatory factors, including high sensitivity c-reactive protein, tumor necrosis factor- α (TNF- α) and IL-6 (5). However, it remains to be elucidated whether BBR can inhibit the abnormally expressed inflammatory factors resulting from pathological alterations in the central nervous system.

In a preliminary study, the authors of the present study have established a mouse model of AD (unpublished). Aberrant expression levels of COX-2, prostaglandin E2 (PGE2), IL-1 β , TNF- α and TNF- α type 1 receptor were observed in neurons of mice with AD exhibiting abnormal behavior. In the present study amyloid β_{25-35} ($A\beta_{25-35}$) was used to induce inflammatory response in the neuroblastoma SH-SY5Y and SK-N-SH cells to model the inflammatory process of patients with AD. The viability of nerve cells and the alterations of mRNA and protein expression levels of inflammatory factors COX-2, PGE2, IL-1 β , TNF- α and TNF- α type 1 receptor were observed prior to and following the treatment. The results of the present study revealed that BBR alleviated the inflammatory response induced by $A\beta_{25-35}$. The mechanism of action of BBR may be associated with the inhibition of proinflammatory mediators.

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Materials and methods

Cells and reagents. Human neuroblastoma SH-SY5Y and SK-N-SH cell lines were purchased from Shanghai Institutes for Biological Sciences (Shanghai, China). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). RNA extraction reagent TRIzol was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA), reverse transcriptase and Taq polymerase were obtained from Promega Corporation (Madison, WI, USA). COX-2 (cat. no. Sc-166475), GAPDH (cat. no. Sc-69778), IL-1 β (cat. no. Sc-32294) and tumor necrosis factor receptor 1 (TNFR1; cat. no. Sc-8436) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). PGE2 and TNF- α ELISA kits were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. BBR was purchased from Nanjing Dulai Biotechnology Co., Ltd. (Nanjing, China).

Cell culture. SH-SY5Y and SK-N-SH cells were maintained in DMEM supplemented with 10% fetal bovine serum (Thermo Fisher Scientific Inc.), 100 U/ml penicillin and 100 U/ml streptomycin in a humidified atmosphere containing 5% CO₂ at 37°C.

Treatment of SH-SY5Y and SK-N-SH cells. SH-SY5Y and SK-N-SH cells were seeded in 6-well plates with 1x10⁶ cells/well. When the cells adhered to the plates, the cell culture medium was discarded and the cells were pre-treated with DMEM medium containing different concentrations of BBR (0, 1 and 10 mol/l) or indomethacin (200 mol/l; Zhuxi Zhongxin Biotech Co., Ltd., Nanjing, China). Cells were incubated for 2 h at 37°C. Subsequently, cells were incubated in DMEM medium with or without A β ₂₅₋₃₅ (5 mol/l) for another 24 h at 37°C. There were five experimental groups: i) Normal control without any treatment; ii) A β ₂₅₋₃₅ model group treated with A β ₂₅₋₃₅ only; iii) A β ₂₅₋₃₅ model + indomethacin group treated with A β ₂₅₋₃₅ + indomethacin (200 mol/l); iv) A β ₂₅₋₃₅ + low-dose BBR group treated with A β ₂₅₋₃₅ + BBR (1 mol/l); and v) A β ₂₅₋₃₅ + high-dose BBR group treated with A β ₂₅₋₃₅ + BBR (10 mol/l). Following the treatment, cells and culture media were collected and centrifuged at 300 x g at room temperature for 2 min for subsequent analysis.

MTT assay on viability of SH-SY5Y and SK-N-SH cells. The SH-SY5Y and SK-N-SH cells at their logarithmic growth phase were collected following 0.125% trypsin digestion and adjusted to a concentration of 1x10⁸ cells/l. Cells were seeded into 96-well plates at a concentration of 10,000 cells/well. After serum starvation for 24 h, the wells were divided into different groups in sextuplicate as mentioned above and 20 μ l MTT (5 g/l) was added to each well. The plates were incubated at 37°C for another 4 h. Subsequently, MTT solution was removed and 150 μ l DMSO was added to each well. The plates were shook for 10 min to dissolve the purple formazan crystals. The light absorbance was measured at a wavelength of 490 nm using a microplate reader. Cell survival rate was calculated using the following equation: Survival rate (%) = $\frac{A_{490\text{ nm}}(\text{experiment})}{A_{490\text{ nm}}(\text{control})} \times 100\%$.

Lactate dehydrogenase (LDH) activity in the cell culture media. Cell culture media were centrifuged at 300 x g at room temperature for 2 min and transferred to the 96-well enzyme-analyzing plates using the LDH kit (Zhuxi Zhongxin Biotech Co., Ltd. Buffer with substrate (50 μ l) was added into each well. The plates were incubated for 30 min at room temperature in the dark and, subsequently, stop buffer (50 μ l) was added into each well. The light absorbance was measured at a wavelength of 490 nm.

PGE2 and TNF- α expression levels determined by ELISA. The microplate was coated with specific antibodies provided in the ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), incubated for 1 h at 37°C and subsequently at 4°C overnight. The plate was washed three times and 200 μ l blocking buffer was added and incubated for 1 h at 37°C. After washing 3 times, cell medium of each group was added and incubated at 37°C for 2 h, and washed three times again. Anti-human immunoglobulin G labeled with horseradish peroxidase was added and incubated at 37°C for 1 h. Stop buffer was added following 3 washes with PBS and 2 with distilled water. The optical density was measured at a wavelength of 450 nm with a microplate reader (model ELX800; BioTek Instrument Inc., Winooski, VT, USA).

COX-2, IL-1 β and TNFR1 mRNA expression levels detected by reverse transcription-quantitative polymerase chain reaction. Total RNA from each group of cells was extracted using TRIzol reagent (Thermo Fisher Scientific, Inc.). Subsequently RNA with A_{260/280} value of 1.80-2.00 was used for reverse transcription. Reverse transcription was performed using 2 μ g of RNA, 1 μ l of random Examer, 1 μ l of RT Superscript at 200 U/l, 10 μ M dNTP and 4% of MMLV reverse transcriptase. Temperature protocol of reverse transcription was as follows: 65°C for 10 min followed by 50°C for 60 min and 85°C for 5 min. The primer sequences were designed using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA) based on the gene sequences from GenBank (6) (Table I). All primers were synthesized by NanJing SunShine Biotechnology Co., Ltd., Nanjing, China. The following thermocycling conditions were used for SYBR Green-Based Real-Time PCR amplification (Invitrogen; Thermo Fisher Scientific, Inc.): Initial denaturation at 95°C for 30 sec; 40 cycles at 94°C for 30 sec, 60°C for 40 sec. The PCR was performed using a thermal cycler (model TP600; Takara Bio, Inc., Otsu, Japan). The Ct values of target genes COX-2, IL-1 β , TNFR1 and control gene GAPDH were calculated. The expression levels of COX-2, IL-1 β and TNFR1 mRNA were calculated using the 2^{- $\Delta\Delta C_q$} method (7).

COX-2, IL-1 β and TNFR1 protein expression detected by western blotting. Cellular proteins were extracted with the RIPA lysis buffer with an EDTA-free protease and a phosphatase inhibitor cocktail tablet (Roche Applied Science, Penzburg, Germany). Proteins were also determined using a BCA assay (NanJing SunShine Biotechnology Co., Ltd.), equal amounts protein/lane (50 μ g) were resolved by 10% SDS-PAGE and electrophoretically transferred onto PVDF membranes. Following incubation in a blocking solution (10% milk) for 2 h at room temperature, the membranes were incubated for 5 h with polyclonal antibodies against COX-2, IL-1 β ,

Table I. Primer sequences used for the PCR.

Gene	Primer sequence (5'→3')		Length of the PCR product (base pair)
	Forward	Reverse	
COX-2	CTGCGCCTTTTCAAGGATGG	CCCCACAGCAAACCGTAGAT	135
IL-1 β	CAGAAGTACCTGAGCTCGCC	AGATTCGTAGCTGGATGCCG	153
TNFR1	CTTCAATTGCAGCCTCTGCC	CTTCCACCGTTGGTAGCGAT	292
GAPDH	GGGAGCCAAAAGGGTCATCA	TGATGGCATGGACTGTGGTC	203

PCR, polymerase chain reaction; COX-2, cyclooxygenase 2; IL, interleukin; TNFR1, tumor necrosis factor receptor 1.

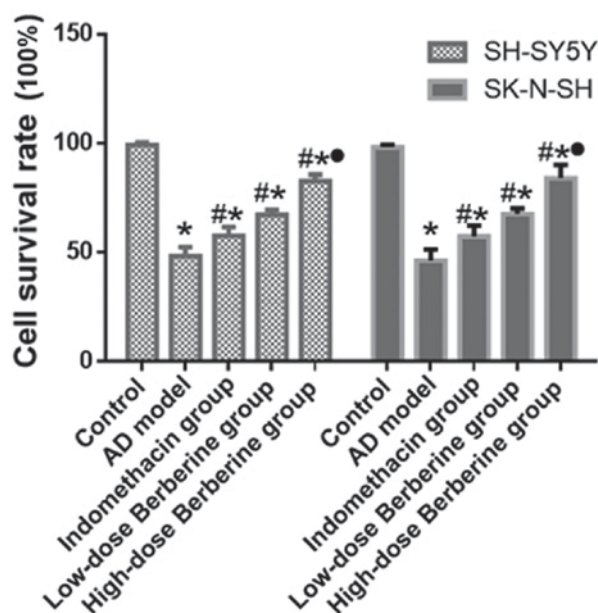


Figure 1. MTT assay on the viability of SH-SY5Y and SK-N-SH cells. Compared with the AD model group, both indomethacin and BBR treatment increased cell survival. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. AD model group; ### $P < 0.05$ vs. low-dose BBR group. BBR, berberine; AD, Alzheimer's disease.

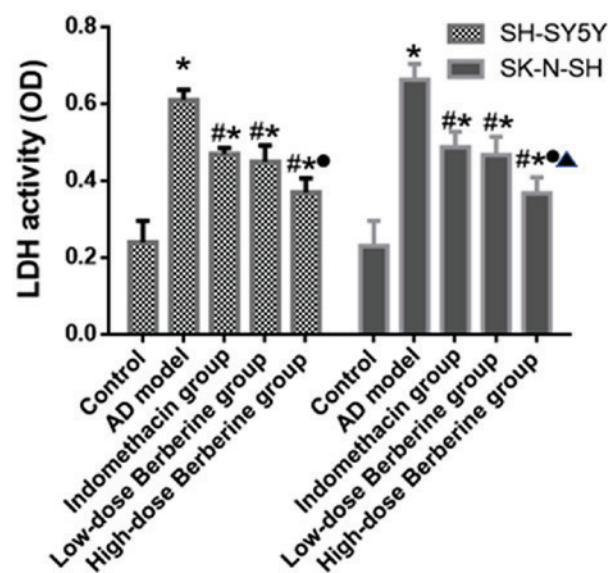


Figure 2. LDH activity of SH-SY5Y and SK-N-SH cells was examined by spectrophotometry. Compared with the control group, the LDH activity of cells treated by $A\beta_{25-35}$ increased significantly. Compared with the AD model group, both indomethacin and BBR treatment reduced the LDH activity of cells. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. AD model group; ### $P < 0.05$ vs. low-dose BBR group. $P < 0.05$ vs. indomethacin group. BBR, berberine; LDH, lactate dehydrogenase; AD, Alzheimer's disease.

TNFR1 and GAPDH at 1:200 dilution at room temperature. The membranes were subsequently washed 3 times in PBS and incubated with horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. Horseradish peroxidase labeled goat anti-mouse Immunoglobulin G secondary antibodies (1:200; cat. no. A25012; Abbkine Scientific Co., Ltd., Wuhan, China) and an enhanced chemiluminescence (ECL) kit were used. The protein-antibody complexes were detected using the ECL detection system. The protein band intensities were evaluated using Scion Image software (Version alpha 4.0.3.2; Scion Corporation, Frederick, MD, USA).

Statistical analysis. All data are presented as the mean \pm standard deviation. Statistical analysis was performed using SPSS software (version 18.0; SPSS, Inc., Chicago, IL, USA). Data were analyzed using one-way analysis of variance followed by Least Significant Difference test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effect of BBR on viability of SH-SY5Y and SK-N-SH cells treated with $A\beta_{25-35}$. Following treatment with $A\beta_{25-35}$, cell viability of the AD model ($A\beta_{25-35}$) significantly decreased compared with the normal control (SH-SY5Y and SK-N-SH cells; both $P < 0.05$). Cell viability increased following treatment with BBR or indomethacin, especially in the high-dose BBR group (SH-SY5Y and SK-N-SH cells; all $P < 0.05$). The results indicated that $A\beta_{25-35}$ induced cellular damage in SH-SY5Y and SK-N-SH cells, and BBR could protect against this damage. Among the experimental groups, high-dose BBR could better protect the damaged cells compared with the indomethacin and low-dose BBR groups (Fig. 1).

Effect of BBR on LDH activity of SH-SY5Y and SK-N-SH cells. The LDH activity of SH-SY5Y and SK-N-SH cells was evaluated in cell culture media. Compared with the respective control

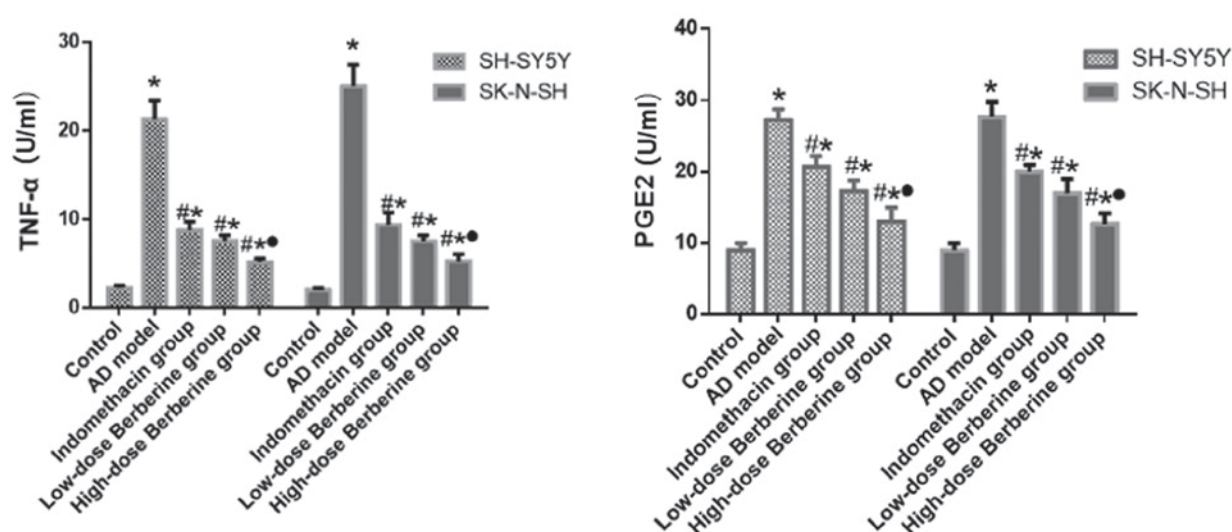


Figure 3. ELISA assay on the levels of PGE2 and TNF- α in SH-SY5Y and SK-N-SH cells. Compared with the control group, PGE2 and TNF- α levels increased following treatment with A β_{25-35} . Both indomethacin and BBR reduced the levels of PGE2 and TNF- α in both SH-SY5Y and SK-N-SH cells. *P<0.05 vs. control group; #P<0.05 vs. AD model group; *P<0.05 vs. low-dose BBR group. BBR, berberine; PGE2, prostaglandin E2; AD, Alzheimer's disease.

groups, the LDH activity of cells induced by A β_{25-35} increased significantly (SH-SY5Y and SK-N-SH cells; all P<0.05). Both indomethacin and BBR reduced the LDH activity of cells compared with the model group (SH-SY5Y and SK-N-SH cells; all P<0.05). High-dose BBR decreased the LDH activity by 39.3% in SH-SY5Y cells and 44.6% in SK-N-SH cells, which was a greater difference compared with that of the low-dose BBR or indomethacin groups (P<0.05; Fig. 2).

Effect of BBR on the expression levels of PGE2 and TNF- α in SH-SY5Y and SK-N-SH cells induced by A β_{25-35} . After SH-SY5Y and SK-N-SH cells were treated with A β_{25-35} , the expression levels of PGE2 and TNF- α secreted into the culture media were examined by ELISA. Compared with the control group, the expression levels of PGE2 and TNF- α increased following A β_{25-35} induction (SH-SY5Y and SK-N-SH cells; all P<0.05). Both indomethacin and BBR reduced the levels of PGE2 and TNF- α in cells (SH-SY5Y and SK-N-SH cells; all P<0.05). High-dose BBR significantly decreased the expression level of PGE2 by 52.4% in SH-SY5Y cells and 54.2% in SK-N-SH cells, compared with the AD model group, which is a greater difference compared with the low-dose BBR or indomethacin groups (P<0.05; Fig. 3). Similarly, high-dose BBR significantly decreased the expression level of TNF- α by 75.6% in SH-SY5Y cells and 78.9% in SK-N-SH cells, which was a greater difference compared with that of the low-dose BBR or indomethacin groups (Fig. 3).

Effect of BBR on mRNA and protein expression of COX-2, IL-1 β , and TNFR1 in SH-SY5Y and SK-N-SH cells induced by A β_{25-35} . Following treatment with A β_{25-35} , the mRNA expression of IL-1 β increased 3.32-fold in SH-SY5Y cells and 3.39-fold in SK-N-SH cells; the protein expression increased 5.86-fold in SH-SY5Y cells and 3.68-fold in SK-N-SH cells. COX-2 mRNA expression increased 5.86-fold in SH-SY5Y cells and 5.6-fold in SK-N-SH cells; protein expression increased 4.04-fold in SH-SY5Y cells and 4.64-fold in SK-N-SH cells. TNFR1 mRNA expression increased 2.93-fold in SH-SY5Y cells and 3.32-fold

in SK-N-SH cells; protein expression increased 4.06-fold in SH-SY5Y cells and 3.02-fold in SK-N-SH cells. These increases have significant difference in AD model compared with control group (P<0.05). Both indomethacin and BBR reduced mRNA and protein expression levels of COX-2, IL-1 β , and TNFR1 (all P<0.05). There were no significant differences in the mRNA and protein expression levels of COX-2, IL-1 β and TNFR1 between indomethacin and low-dose BBR groups (P>0.05), however, there was a significant difference in the mRNA and protein expression level of COX-2, IL-1 β and TNFR1 treated by high-dose BBR compared with the low-dose BBR group (all P<0.05; Figs. 4-6).

Discussion

Previously, studies on the pathogenesis of AD revealed that chronic inflammation of the central nervous system is one of the important pathological features, along with the phosphorylation of tau protein, senile plaques and amyloidosis (8-10). It was reported that factors that participate in the pathophysiological process of AD include cytokines, such as IL-1, TNF- α and transforming growth factor β . It has been hypothesized that pathogenesis of AD may be initiated by up-regulating the expression of amyloid precursor protein by the stimulation of its promoter (11). Increased expression levels of amyloid precursor protein result in up-regulated expression of acetylcholinesterase (AChE) and increased AChE activity (12). These alterations may further induce malnutritional axonal growth and increase the level of phosphorylated tau protein (13). Finally, activation of astrocytes increases the level of inflammatory factors including TNF- α (14).

In the central nervous system, neurons and microglial cells normally exhibit no or very low levels of inflammatory factors (1). In chronic neurodegenerative diseases, the high level of inflammatory factors in the central nervous system may result from the invasion of peripheral lymphocytes and exogenous inflammatory factors or be induced by amyloid deposits (1). Peripheral lymphocytes and inflammatory factors

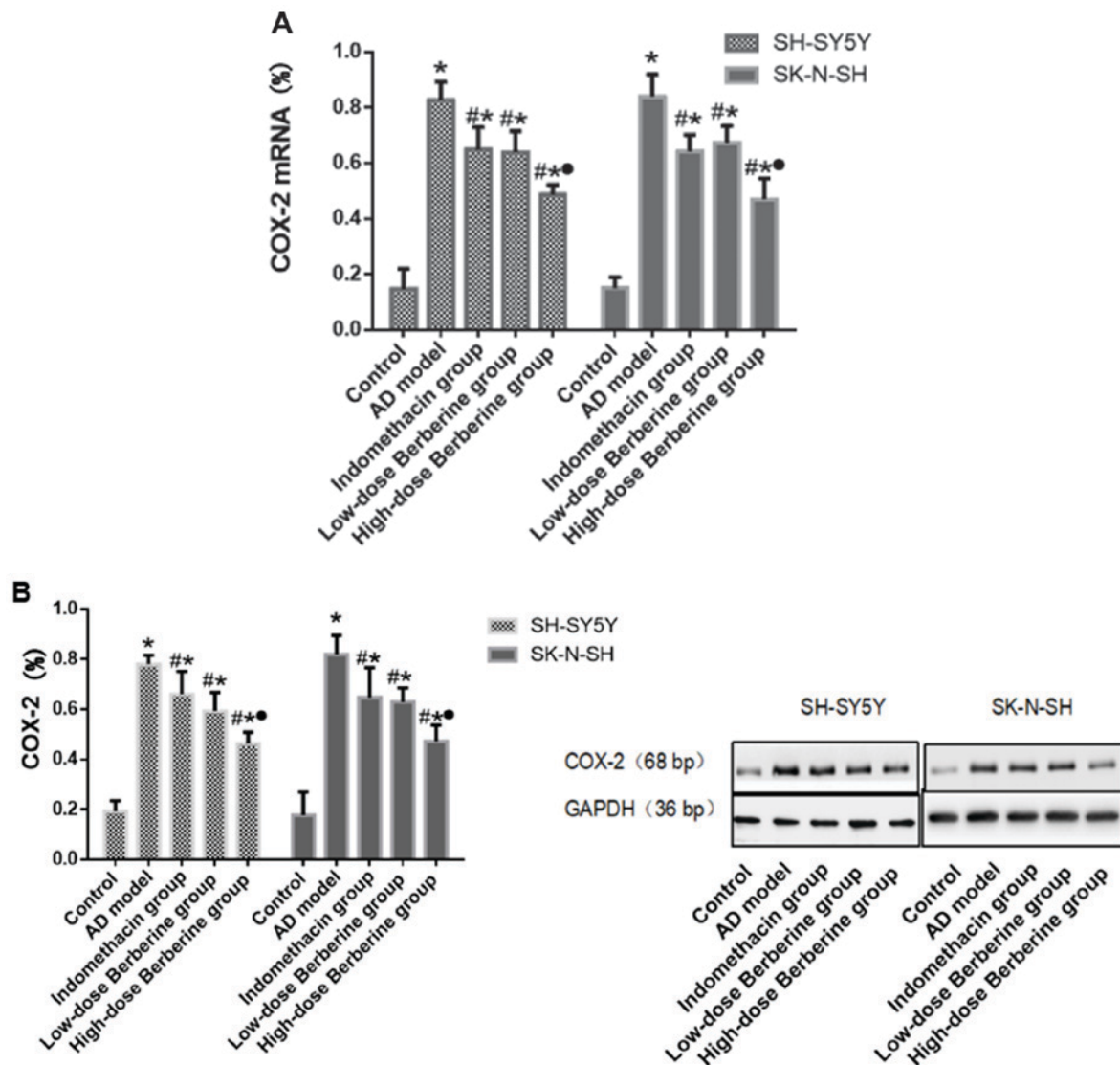


Figure 4. Expression of COX-2 mRNA and protein. (A) RT-PCR analysis of the mRNA expression of COX-2 in SH-SY5Y and SK-N-SH cells. BBR and indomethacin inhibited $A\beta_{25-35}$ -induced COX-2 mRNA expression. (B) Western blot analysis of the protein expression of COX-2 in SH-SY5Y and SK-N-SH cells. BBR and indomethacin inhibited $A\beta_{25-35}$ -induced COX-2 protein expression. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. AD model group; • $P < 0.05$ vs. low-dose BBR group. COX-2, cyclooxygenase 2; BBR, berberine; AD, Alzheimer's disease.

are not the primary causes of inflammation of the central nervous system due to the blood-brain barrier (15). The main cause of the neurodegenerative process in AD is neuronal inflammation induced by abnormal deposition of amyloids in the brain (16). It has been demonstrated that interaction between amyloid β and its receptors resulted in intracellular signal transduction and activation of microglial cells to generate inflammatory factors (17). NSAIDs induce a protective effect by inhibiting inflammatory factors (18).

A small clinical trial revealed that oral administration of low dose (0.4 g) BBR 3 times/day can inhibit the levels of inflammatory factors (5). BBR can cross the blood brain barrier and directly affect the cerebral cortex and hippocampus (19). BBR exhibited a protective effect and therapeutic potential for chronic brain injury induced by aluminum overload and other diseases of the central nervous system in mice (20,21). It remains to be elucidated whether BBR could attenuate the inflammatory reaction induced by AD in the central nervous system. In the present study, $A\beta_{25-35}$ was used

to induce inflammatory reaction in SH-SY5Y and SK-N-SH cells, to model the inflammatory response of AD. The results indicated alterations of cell viability and expression levels of inflammatory factors including COX-2, PGE2, IL-1 β , TNF- α and TNF- α type 1 receptor following treatment with BBR.

Nerve cells and neural glial cells normally express low levels of COX-2 and IL-1 β for basic brain function, including synaptic plasticity and memory enhancement. The neuronal membrane excitability is modulated by adrenergic, noradrenergic and glutamatergic neurotransmitters (1). Under pathological conditions nerve cells may be affected by ischemia, hypoxemia, mitogens, cytokines and hormones. In these cases, the levels of inflammatory factors in nerve cells increase, leading to neuronal degeneration (16). High level of COX-2 increases the synthesis of PGE2 which can induce cell apoptosis, damage the sulfhydryl group of intracellular proteins and cause neurodegenerative disorders (22). IL-1 β is the primary factor contributing to the formation of senile plaques in AD. Overexpression of IL-1 β in microglia is a characteristic feature

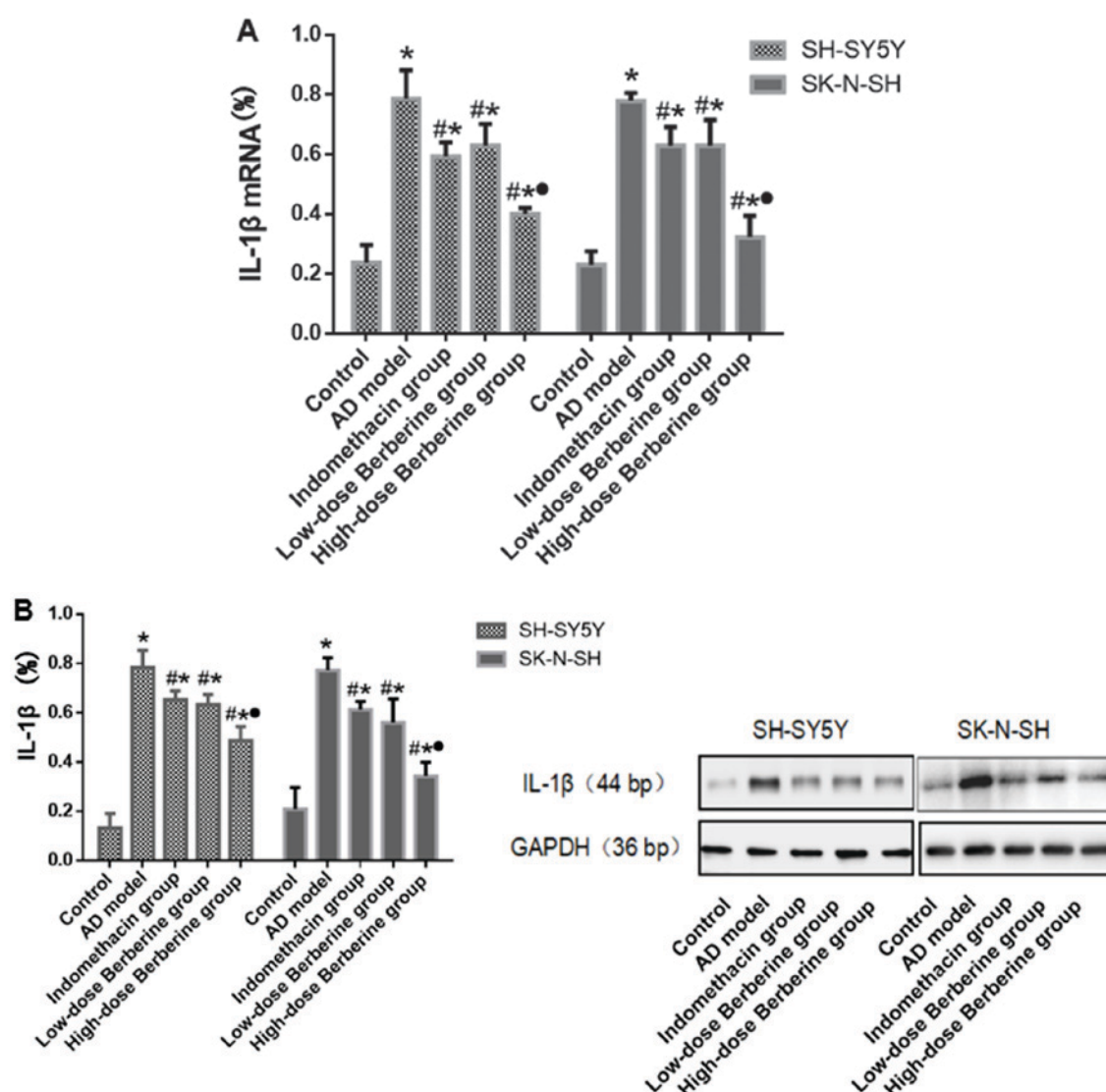


Figure 5. Expression of IL-1 β mRNA and protein. (A) RT-PCR analysis of the mRNA expression of IL-1 β in SH-SY5Y and SK-N-SH cells. BBR and indomethacin inhibited A β_{25-35} -induced IL-1 β mRNA expression. (B) Western blot analysis of the protein expression of IL-1 β in SH-SY5Y and SK-N-SH cells. BBR and indomethacin inhibited A β_{25-35} -induced IL-1 β protein expression. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. AD model group; ## $P < 0.05$ vs. low-dose BBR group. BBR, berberine; IL, interleukin; AD, Alzheimer's disease.

of AD. It has been shown *in vitro* that IL-1 β does not alter cell morphology, number and activity (23). However, the combined effect of IL-1 β and A β_{25-35} exacerbated the cytotoxicity of A β_{25-35} in a time- and dose-dependent manner. The mechanism of action is associated with overexpression of mRNA induced by the regulating sequences of the 5' promoter region of the APP gene (24). Therefore, drugs inhibiting COX-2 and IL-1 β expression can antagonize the inflammatory reaction in the central nervous system to protect nerve cells.

TNF- α is a pleiotropic cytokine (25). In addition to induction of neuronal cytotoxicity, TNF- α can increase the permeability of blood-brain barrier, which promotes lymphocyte infiltration (26) and up-regulates the expression of intercellular adhesion molecule 1 (ICAM-1). The biological function of TNF- α is to transmit signals through two cell surface receptors including TNFR1 and TNFR2 (25). Gene knockout studies and experiments using receptor agonist antibodies indicated that these two receptors react with different proteins and activate different signal transduction

pathways (27-29). Following the binding of TNF- α and TNFR1, the mitogen-activated protein kinase, protein kinase C, and protein kinase A activate nuclear factor κ B, which promotes transcription of numerous proinflammatory cytokines (30). The binding of TNF- α and TNFR1 participates in transcriptional regulation of inflammatory genes. It serves a role in mediating inflammation in the central nervous system (31). A previous study revealed that TNFR1 blocking peptide binds to TNFR1 and antagonizes the pro-inflammatory effect of TNF- α (32). Therefore, it can be hypothesized that inhibiting the expression of COX-2 and IL-1 β can antagonize the inflammatory reaction in the central nervous system to protect nerve cells.

In the AD group of the present study, the decrease in cell survival and increase in LDH activity of SH-SY5Y and SK-N-SH cells indicated that the cells were severely damaged by A β_{25-35} . Following BBR drug intervention, cell survival increased and LDH activity decreased, indicating that BBR reduced the cytotoxicity of A β_{25-35} . The mechanism may be

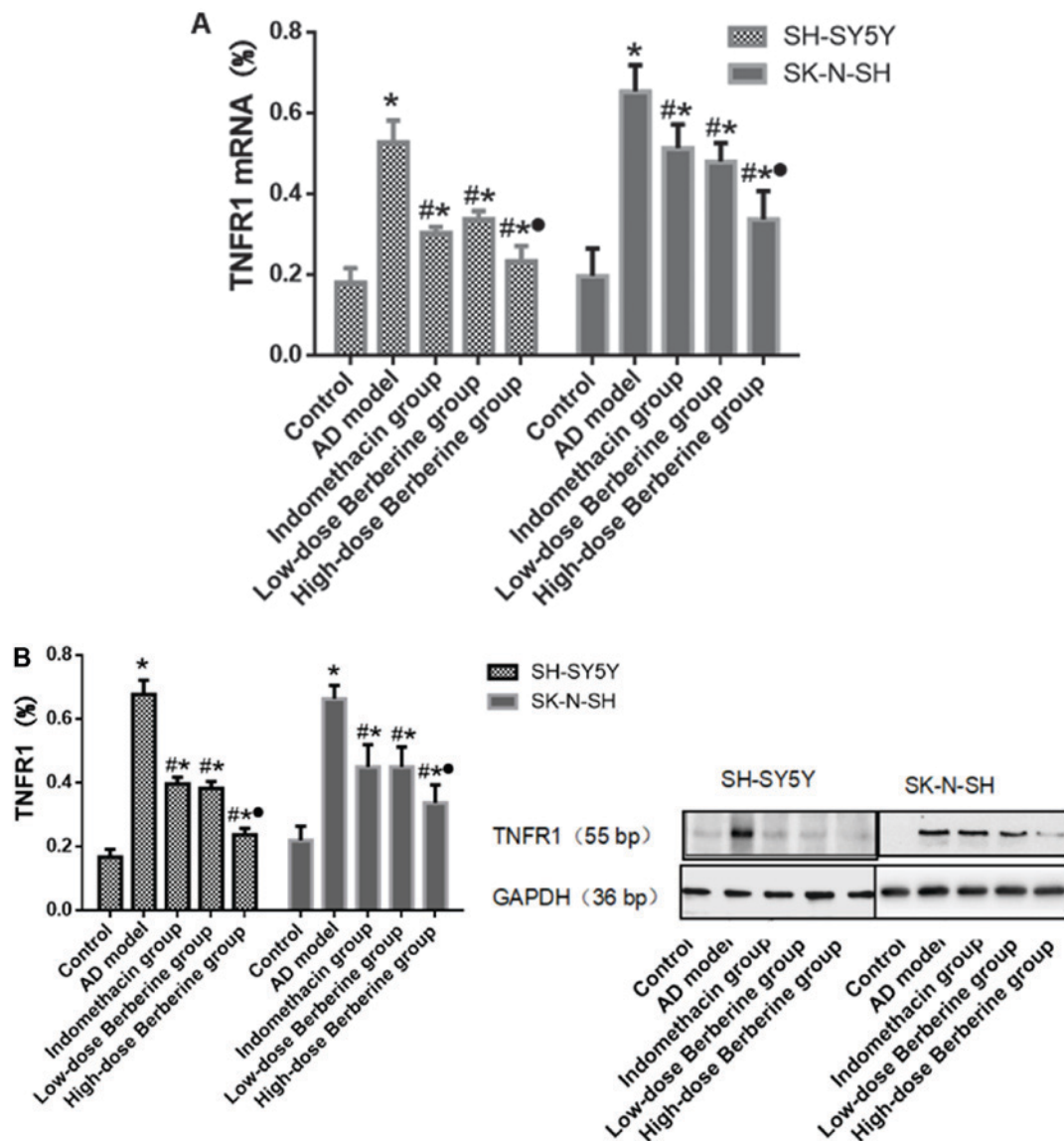


Figure 6. Expression of TNFR1 mRNA and protein. (A) RT-PCR analysis of the mRNA expression of TNFR1 in SH-SY5Y and SK-N-SH cells. BBR and indomethacin inhibited $A\beta_{25-35}$ -induced TNFR1 mRNA expression. (B) Western blot analysis of the protein expression of TNFR1 in SH-SY5Y and SK-N-SH cells. BBR and indomethacin inhibited $A\beta_{25-35}$ -induced TNFR1 protein expression. * $P < 0.05$ vs. control group; ** $P < 0.05$ vs. AD model group; *** $P < 0.05$ vs. low-dose BBR group. TNFR1, tumor necrosis factor receptor 1; BBR, berberine; AD, Alzheimer's disease.

associated with the down-regulation of expression of TNF- α , COX-2, IL-1 β and TNFR1. Compared with the low-dose BBR and indomethacin groups, high dose BBR significantly decreased the expression of LDH, PGE2, TNF- α , COX-2, IL-1 β and TNFR1. The results suggested that BBR may regulate PGE2 through other pathways in addition to the inhibition of the COX-2 signaling. The high expression levels of PGE2 may mediate brain tissue damage in several ways including: i) Increased adhesion of platelets and neutrophils to vascular endothelial cells (33); ii) increased permeability of the blood-brain barrier (34); iii) cytotoxicity mediated by excitatory amino acids (35); and iv) increased generation of oxygen free radicals and the toxic effect of nitric oxide leading to decreased cell viability.

Taken together, the present study suggested that BBR can protect nerve cells against inflammatory response mediated by $A\beta_{25-35}$. The mechanism of action may be associated with down-regulation of PGE2, COX-2, IL-1 β , TNF- α and TNFR1.

High-dose BBR exhibits a marked inhibitory effect on the inflammatory response and could potentially be used for the treatment of AD.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HZ and LY conceived and designed the study. JX and WW performed the experiments and wrote the paper. LY reviewed and edited the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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