Ameliorating Ribosylation-induced A β Pathology by Berberine via Inhibiting mTOR/p70S6K Signaling

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Research

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Abstract

Background: Berberine (BBR) plays a neuroprotective role in the pathogenesis of Alzheimer's disease (AD), inhibiting β -amyloid ($A\beta$) production and promoting $A\beta$ clearance. Advanced glycation end products (AGEs) promote $A\beta$ aggregation and tau hyperphosphorylation. The activation of mTOR signaling occurring at the early stage of AD has a prominent impact on the $A\beta$ production. This work focused on whether BBR regulates the production and clearance of ribosylation-induced $A\beta$ pathology via inhibiting mTOR signaling.

Methods: Western Blot and Immunofluorescence staining were used to detect the related proteins of the mammalian target of Rapamycin(mTOR) signaling pathway and autophagy, as well as the related kinases of $A\beta$ generation and clearance. Tissue sections and Immunofluorescence staining were used to observe $A\beta_{1-42}$ in APP/PS1 mice hippocampal. Morris water maze (MWM) test was used to measure the spatial learning and memory of APP/PS1 mice.

Results: BBR improves spatial learning and memory of APP/PS1 mice. BBR limits the activation of mTOR/p70S6K signaling pathway and enhances autophagy process. BBR reduces the activity of BACE1 and γ -secretase induced by D-ribose, and enhances $A\beta$ -degrading enzymes and Neprilysin, and inhibits the expression of $A\beta$ in APP/PS1 mice.

Conclusions: BBR ameliorates ribosylation-induced $A\beta$ pathology via inhibiting mTOR/p70S6K signaling, and improves spatial learning and memory of the APP/PS1 mice.

Background

Alzheimer's disease (AD) is an age-related and insidious neurodegenerative disease that is clinically characterized by progressive cognitive decline accompanied by language, behavioural disorders, social dysfunction, and eventually death (1). Depending on the age of onset, AD is divided into presenile dementia that before 65 years old and senile dementia that after 65 years old (2). The main pathological features of AD includes the senile plaques (SP) formed by the aggregation and deposition of β -amyloid peptides ($A\beta$) which is cleaved from amyloid precursor protein (APP), and neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau protein, as well as loss of synapses in neocortex and hippocampus, neuroinflammation, oxidative stress and nerve cell death (3, 4). For many years, AD has become a research hotspot in the neuroscience scientific community. Evidence from clinical and basic studies on $A\beta$ has pointed out that it plays a key role in the onset and development of AD pathogenesis. However, there have been no mature schemes or effective drugs targeted at $A\beta$ for the treatment of AD, suggesting the complexity and challenge of the $A\beta$ hypothesis (5).

In recent years, the role of non-enzyme catalyzed AGEs in the pathogenesis of AD has been drawn more and more attention. AGEs can bind to advanced glycation end products receptor (RAGE) on the surface of nerve cells, and then AGEs and RAGE interact with glial cells to promote $A\beta$ aggregation and tau hyperphosphorylation (6). Studies have found that D-ribose-induced ribosylation and excessive AGEs

production play an important role in tau hyperphosphorylation and the NFTs formation (7). Still and all, the underlying mechanism of abnormal glycosylation in the pathogenesis of AD remains unclear.

Autophagy is an important mechanism of intracellular recycling that delivers cellular metabolize material to lysosomes for degradation, in response to various forms of stress such as nutrient deficiency, organelle damage and protein misfolding (8). According to the process mechanism, autophagy has been identified in three types: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). As the most common form, macroautophagy is characterized by the formation of autophagosome after the double membrane structure wraps the substrate protein, and autophagosome fuses with lysosome to form autolysosome, which releases the substrate protein into lysosome and eventually degrades it under the action of hydrolase, so as to achieve cell homeostasis and organelle renewal (9, 10).

Increasing studies have shown that autophagy is closely related to the occurrence and development of neurodegenerative diseases such as AD, Parkinson's disease (PD) and Huntington's disease (HD) (11). Emerging evidence suggests a complex interaction between autophagy and A β , which may contribute to the progression of AD. Nilsson and Saido reported that autophagy plays a key role in the A β plaque formation, while autophagy deficiency results in intracellular A β accumulation and memory impairment of AD mice (12, 13). The defective autophagy–lysosome pathway not only leads to A β accumulation, but also hinders its degradation. A study found that estrogen receptor β (ER β) interacting with autophagy related protein (ATG) 7 enhanced autophagy activity, promotes A β clearance in vitro (14). Other three essential components of autophagy, ATG5, Beclin1, and Ulk1 were also proved to involve in the degradation of A β (15).

Berberine (BBR), a natural alkaloid that can be extracted from the roots, rhizomes, stems, and bark of some medicinal plants such as Huanglian (*Coptis chinensis*), *Berberis aristata*, *Hydrastis canadensis*, *Tinospora cordifolia*, *Phellodendron amurense* and so on. As a traditional Chinese medicine with a long history of medicinal use, BBR is widely used for treating diarrhea, gastroenteritis and type II diabetes (16, 17). Furthermore, BBR possesses the properties of anti-cancer (18, 19), anti-gynecopathy (20), anti-cardiovascular disease (21) and anti-neurodegenerative disease (22). A series of studies have focused on BBR and the molecular pathogenesis of AD. BBR could reduce the accumulation of A β by activating AMP-activated protein kinase in N2a/APP695sw cells (23), alleviate A β induced mitochondrial dysfunction and synaptic loss in primary cultured hippocampal neurons (24). In addition, BBR improves cognitive impairment by promoting autophagic clearance and inhibiting production of A β in APP/Tau/PS1 mouse (25).

Our previous studies have shown that BBR inhibits the activity of β / γ -secretases, enhances α -secretases, and lowers the A β level in the hippocampus of AD mice, and improves Alzheimer's-like cognitive impairment, but whether this effect is through the autophagy pathway has not been studied (26). In this study, N2a cells and transgenic APP/PS1 mice were used to study the regulation of BBR on autophagy and A β aggregation, and we verified the hypothesis that BBR improves the symptoms of AD by regulating the autophagy pathway. This study focused on whether BBR regulates the production and clearance of

ribosylation-induced A β pathology via regulating mTOR signaling and autophagy pathway, using N2a cells and transgenic APP/PS1 mice.

Methods

Drugs and animals

BBR (100 mg/tablet) was purchased from Sanofi Minsheng Health Pharmaceutical Co. Ltd (Hangzhou, China). D-ribose was purchased from Sigma-Aldrich (St. Louis, MO, USA) (V900389). Aminoguanidine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA) (396494). Rapamycin was purchased from Cell Signaling Technology, Lnc (CST) (MA, Boston, USA) (9904). APP/PS1 transgenic mice were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences & Peking Union Medical College.

Morris water maze test

An escape platform (diameter 8 cm) was placed 1 cm below the surface in the northeast quadrant of a circular tank (diameter 1.2 m and depth 0.4 m, 25 $\pm$ 1 $^{\circ}$ C). Mice adapted to the experimental environment were trained 4 times per day for 6 consecutive days as acquisition trials. Each trial began with putting a mouse into the different quadrant and letting it keep swimming freely for 60 s. Each mouse climbed onto the platform and staid for 5 s (or failed to locate the platform within 60 s was given extra 30 s to be guided to the platform), they were put back in their cages and wiped dry. The time taken for each mouse to reach the platform is considered the escape latency, which together with swimming trajectory were recorded by the ANY-maze video tracking software (Stoelting Co., USA). After 1 day's rest, the mice were performed with probe test without the platform for 60 s. The mice were placed into the quadrant opposite to the original platform quadrant. The time, distance in the original platform quadrant and the number of crossings that occurred in the probe were observed and recorded.

Cell culture and treatments

Mouse neuroblastoma cells, Neuro-2a (N2a) were kindly provided by Professor Tang Yong at Basic Medical Science College of Chongqing Medical University (Chongqing, China). N2a cells were cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F12, Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Invigentech, Irvine, CA, USA) and antibiotics (100 U/ml penicillin and 100 μ g/ml streptomycin), incubated in humidified atmosphere with 5% CO₂ at 37 $^{\circ}$ C. N2a cells were treated with 10 mM D-ribose for 24 h, used for constructing hyperribosylation model. For evaluate the effect of BBR on autophagy activity, N2a cells were precoated with 1 μ M BBR for 12 h, while the control group was treated with 1.5 mM aminoguanidine hydrochloride (AGEs inhibitor) for 12 h after D-ribose tresatment, or 100 nM Rapagymmin for 12 h.

Western blot analysis

Cell lysates and the brain tissue of APP/PS1 mice were lysed in RIPA Lysis Buffer containing protease inhibitor PMSF. Extracted total protein was determined by BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China), and equivalent protein were separated on the corresponding concentration of gels and then transferred to PVDF membranes (Bio-Rad, Hercules, CA, USA). Membranes were blocked and incubated primary antibodies against Phospho-mTOR (Ser2448) (CST, 5536), mTOR (CST, 2983), Phospho-p70 S6 Kinase (Thr389) (CST, 9234), p70 S6 Kinase (CST, 9202), SQSTM1/p62 (CST, 39749), LC3A/B, Beclin-1, ATG3 (Autophagy Antibody Sampler Kit, CST, 4445), LC3B (CST, 3868), BACE (CST, 5606), AGE (abcam, 23722), Presenilin 1 (abcam, 76083), Presenilin 2 (abcam, 51249), Neprilysin (proteintech, 18008) overnight at 4 °C. Then membranes were incubated with secondary antibody for 1 h at room temperature. Protein bands were visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore Corporation, Billerica, MA, USA) and Tanon-5200 multi chemiluminescence analysis system (Tanon, Shanghai, China).

Paraffin section

The whole brains of APP/PS1 mice were removed and fixed with 4% paraformaldehyde for 48 h. The specimen was cut into 2 µm paraffin sections using a Rotary Microtome (HM 340E, Thermo Scientific) along the long axis in sagittal plane. After deparaffinization, antigen retrieval was performed in citric acid solution through microwave oven. Then paraffin sections were used for immunostaining analysis.

Immunofluorescence staining

N2a cells cultured on coverslips were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 in PBS. N2a cells or 2 µm paraffin sections were blocked with 5% goat serum albumin, and incubating with primary antibodies Phospho-mTOR (Ser2448), LC3B, BACE1, Presenilin 2 or β-Amyloid 1–42 (abcam, 10148) at 4 °C overnight. After incubation with secondary antibodies Alexa Fluor® 488-conjugated antibodies (ZSGB-BIO, Beijing, China) for 1 h in the dark, the nucleuses were stained using DAPI (Beyotime). The coverslips were installed onto glass slides using Antifade Mounting Medium (Beyotime), and a laser scanning confocal microscope (Leica TCS-SP2, Germany) or NEXCOPE microscope (NE900, USA) was used for detection.

Statistical analyses

All the experimental data were analysed using GraphPad Software. ANOVA and Independent-sample t test were applied to compare the differences of measurement data between each group. The data were represented as means ± standard error of mean (SEM) and $p < 0.05$ was considered statistically significant.

Results

BBR promotes autophagy by inhibiting the activation of mTOR signaling pathway in N2a cells.

To examine the effect of BBR on mTOR and autophagy signaling, Rapamycin was used as a positive control, which stimulates autophagy by inhibiting mTOR. N2a cells were treated with 1 µM BBR or

100 nM Rapamycin for 24 h. After exposure to BBR, the level of p-mTOR and p-p70S6K decreased compared to the control group, which was the same effect as the treatment with Rapamycin (Fig. 1a, b). Similarly, after BBR treatment, the expression of autophagy-related proteins LC3B, ATG3 and Beclin1 significantly increased, synchronously, the elective autophagy receptor SQSTM1/p62 was degraded (Fig. 1c, d). Then we verified these results using immunofluorescence. As illustrated in Fig. 1e, compared with control group, BBR treatment group showed the enhanced fluorescence intensity of both p-mTOR and LC3B proteins, which was consistent with the effect of Rapamycin.

BBR decreases BACE1, γ -secretase and enhances A β -degrading enzymes Neprilysin in N2a cells.

We further studied the effect of BBR on A β associated kinase in N2a cells. As shown in Fig. 2a, b, compared with the control group, BBR treatment inhibited the expression of β -secretase, BACE1 and γ -secretase, PS1 and PS2, and enhanced the expression of A β decomposition enzyme, Neprilysin. This effect was similar to Rapamycin. Next, immunofluorescence staining was used to detect the effect of BBR on β -secretase, BACE1 and γ -secretase, PS2. As shown in Fig. 2c, BBR treatment decreased the fluorescence intensity of BACE1 and PS2 proteins, which was consistent with the effect of Rapamycin.

BBR counteracts the activation of mTOR signaling pathway, the inhibition of autophagy, the increase of BACE1, γ -secretase and the decrease of Neprilysin induced by D-ribose in APP/PS1 mice.

It has been reported that AGEs can bind with RAGE and then interact with glial cells to promote the aggregation of A β and the hyperphosphorylation of tau protein. D-ribose inducing ribosylation and the generation of excessive AGEs are an important cause of tau hyperphosphorylation and the formation of NFTs. To clarify the mechanism by which BBR regulating mTOR signaling pathway to promote autophagy, we first analyzed the effect of BBR and D-ribose on mTOR signaling pathway. D-ribose intraperitoneal injecting increased the expression of AGE, p-mTOR and p-p70S6K protein in APP/PS1 mice brain. When mice injected with D-ribose were given BBR intraperitoneally, the increase of AGE, p-mTOR and p-p70S6K protein expression was inhibited, which was similar to the effect of AGE inhibitors (Fig. 3a, b). Then we examined the effect of BBR and D-ribose on autophagy-related proteins. D-ribose intraperitoneal injecting decreased the expression of Beclin1, ATG3 and LC3B, and increased p62 protein in APP/PS1 mice brain. After additional intraperitoneal administration of BBR, the decrease of Beclin1, ATG3 and LC3B, and the increase of p62 protein expression was inhibited, this was consistent with the the effect of AGE inhibitors (Fig. 3c, d). Then we investigated the effect of BBR on A β associated kinase in APP/PS1 mice. As shown in Fig. 3e, f, compared with the control group, D-ribose treatment increased the expression of β -secretase, BACE1 and γ -secretase, PS1 and PS2, and decreased the expression of A β decomposition enzyme, Neprilysin. When the D-ribose treated mice were given BBR intraperitoneally, the expression of BACE1, PS1 and PS2 reduced significantly, concurrently, the expression of Neprilysin increased dramatically.

BBR improves spatial learning and memory and inhibits the A β ₁₋₄₂ expression of APP/PS1 mice.

The spatial learning and memory of APP/PS1 mice was performed using the MWM test followed the schematic diagram of experimental process (Fig. 4a). On the 5 days space navigation training, the four experimental groups all showed a time dependent decrease of escape latency. Compared with the D-ribose supplementary AGE inhibitor group and the D-ribose supplementary BBR group, the D-ribose treatment group had longer escape latency from day 2 to day 5 ($p \leq 0.05$) (Fig. 4b). After removing the platform, the time in the platform location, the times of across platform and the retention time in the target quadrants of D-ribose treatment group had significantly decreased compared with control group, which was abrogated in the D-ribose supplementary AGE inhibitor group and the D-ribose supplementary BBR group (Fig. 4c, d, e). These differences were visualized in the representative mouse trajectories taken by APP/PS1 mice during the space exploration task (Fig. 4f). Immunofluorescence staining was used to intuitively observe the effect of BBR on A β in APP/PS1 brain. Compared with D-ribose group, BBR treated group showed significant decreases in the immunoreactive region of A β_{1-42} in APP/PS1 mice hippocampal (Fig. 4g). These results indicated that BBR improved spatial learning and memory, promoted the degradation of A β_{1-42} in the hippocampus of APP/PS1 mice.

Discussion

BBR have been reported to play a neuroprotective role in the pathogenesis of AD, inhibiting A β production and promoting A β clearance. This study has demonstrated that BBR improves spatial learning and memory of APP/PS1 mice. BBR promotes autophagy by inhibiting the activation of mTOR/p70S6K signaling pathway, reduces the activity of BACE1 and γ -secretase induced by D-ribose, and enhances A β -degrading enzymes, Neprilysin. Thus, BBR regulates autophagy through mTOR signaling pathway, and inhibits ribosylation-induced A β production and promotes A β clearance (Fig. 5).

Amyloid plaques resulting from the deposition of A β peptide are the typical hallmark of AD pathology. In the amyloidogenic pathway, transmembrane protein APP is cleaved by α -secretase, β -secretase (BACE1) and γ -secretase which mainly contains four subunits: presenilin (PS1, PS2), nicastrin, APH-1 and PEN-2 (27). α -Secretase cleaves APP producing a soluble α -fragment (sAPP α) and a 83 residue C-terminal fragment bound to membrane (C83), which will be cleaved by γ -secretase to a peptide fragment called P3 and a APP intracellular domain (AICD). This process is called nonamyloidogenic pathway. On the contrary, in the amyloidogenic pathway, BACE1 cleaves APP, releasing a soluble β -fragment (sAPP β) and a C-terminal fragment (C99), which will be processed by γ -secretase and lead to the formation of A β and AICD (28, 29).

BBR is a natural isoquinoline alkaloid isolated from *Rhizoma coptidis* and other herbs, whose working mechanism still needs to be further explored. Studies have shown that BBR has the therapeutic potential for central nervous system (CNS) diseases, such as AD, cerebral ischemia, mental depression, schizophrenia and anxiety (30). A series of in-depth studies have been carried out on animal models of AD or in vitro experiments. In HEK293/tau cells, BBR attenuates calyculin A induced cytotoxicity and tau hyperphosphorylation (31). Durairajan et al demonstrated that BBR ameliorates A β pathology, gliosis, and cognitive impairment in AD transgenic mouse model (32). Moreover, BBR against the altered intrinsic

properties of the CA1 neurons induced by A β neurotoxicity in A β treatment rats (33). Panahi et al reported that BBR treatment could restore Al maltol-induced behavioral derangements by regulating the physiological abilities, histological changes and BACE1 activity in the rabbit model of AD (34). In addition, as an autophagy regulator, BBR might be able to regulate autophagy in response to the pathological mechanisms of various diseases (35). Our previous research found that BBR inhibits the activity of β / γ -secretases, enhances α -secretases, lowers the A β level and improves cognitive impairment of AD mice (26). In this report, we demonstrated that BBR decreases the expression of β -secretase, BACE1 and γ -secretase, PS1 and PS2, enhances A β -degrading enzymes Neprilysin in N2a cells and AD mice.

Autophagy plays an important role in the pathogenesis of AD. A large number of studies have found that the autophagic lysosomal pathway obstacle not only leads to the production of toxic A β , but also is an important reason for the failure of the clearance of A β , finally leading to the accumulation and deposition of A β in the brain (36). Furthermore, autophagy is considered to be another degradation pathway of A β , which is similar to the A β degradation kinase (37). Autophagy is controlled by a highly regulated process. mTOR is a highly conserved serine/threonine kinase that controls cell growth and proliferation mainly by regulating the metabolism of amino acids, glucose, nucleotides, fatty acids and lipids, and by suppressing autophagy (38). mTOR activates its downstream serine/threonine kinase p70 ribosomal S6 kinase (p70S6K) in response to intracellular state change (39). Studies have found that mTOR pathway is not only involved in regulating autophagy termination and lysosomal recombination in NRK cells (40), but also considered as an essential homeostatic protein for interneuron development in the brain (41). Beclin1 is another key regulator of autophagy, which regulates the synthesis and maturation of autophagosome (42). During the formation of autophagosome, microtubule-associated proteins light chain 3A (LC3A) is activated by autophagy related protein 7 (ATG7), transferred to ATG3, then conjugated with phosphatidyl ethanolamine to form LC3B. SQSTM1/p62 is a key factor in the formation of autophagosome and is selectively degraded in autolysosome system (43). Our recent study has found that autophagy disorder enhanced the production of A β by enhancing the activity of the γ -secretase complex. Autophagy inhibitor could significantly activate the expression of PS1, Nicastrin and pen-2 in the γ -secretase and promote the production of A β (44). In this study, we first examined whether BBR promoted autophagy by regulating the mTOR signaling pathway, thereby reducing the expression of A β and its related kinases of APP/PS1 mice and N2a cells. Our results indicate that BBR reduced the activation of mTOR/p70S6K, promoted autophagic lysosomal pathway and followed by reducing A β generation in APP/PS1 mice and N2a cells.

AGEs has been evidenced as a major source of neurotoxicity in AD via promoting A β aggregation and tau hyperphosphorylation. AGEs interacting with RAGE is associated with AD pathology and regulates APP processing through increasing the expression of cathepsin B and asparagine endopeptidase (AEP), or conjunction with the reactive oxygen species (ROS), which increased A β formation (45, 46). In this study, D-ribose caused the overexpression of AGEs, promoted the intensity of mTOR signaling pathway, and inhibited autophagy. Then we detected whether BBR regulates the expression of AGEs and thus influences the mTOR signaling pathway and autophagy. After adding BBR, the express of AGEs and mTOR/p70S6K signaling were inhibited, autophagy was enhanced. In addition, BBR also counteracted the increase of BACE1, γ -secretase and the decrease of Neprilysin induced by D-ribose. Furthermore, we

intuitively detected the effect of BBR on expression of A β in the hippocampus of D-ribose treated mice. The results showed that BBR significantly inhibited the signal intensity of A β . Moreover, MWM experiment showed that BBR improved cognitive impairment of AD mice. However, the presence of other transcription factors underlying AGEs and autophagy interacting to induce the downregulation of A β remains unknown, and the factors through which BBR exerts its effect should be further investigated.

Ribosylation-induced A β pathology has been well-accepted across all animal species, and it is therefore expected that seeking a selective inhibitor limiting ribosylation-induced A β pathology will be an important defense against internal A β pathological injury. The discovery provides a powerful pathway to help to meet these needs, which BBR ameliorates ribosylation-induced A β pathology via inhibiting mTOR/p70S6K signaling.

Limitation

One limitation of our study should be noted that only APP/PS1 mice and N2a cells were examined, which can not be directly extrapolated to humans. Furthermore, in addition to the MWM test, we didn't adopt more behavioral tests to assess cognitive function, which may mask the specific cognitive function effects of BBR on AD mice. Notwithstanding its limitation, this study does suggest that BBR could ameliorate ribosylation-induced A β pathology via inhibiting mTOR/p70S6K signaling and enhancing autophagy. Using more behavioral tests means to monitor the effect of BBR on the cognitive function of AD mice, and exploring the clinical mechanism of BBR in alleviating A β pathology will be the focus of our future research.

Conclusions

In this study, we show that BBR restrains A β generation in the APP/PS1 mice and N2a cells. Firstly, BBR suppresses mTOR/p70S6K signaling and promotes autophagic lysosomal pathway, and promotes A β clearance. Moreover, BBR also inhibits ribosylation-induced A β generation in APP/PS1 mice and N2a cells. Together, these findings reveal ameliorating ribosylation-induced A β pathology by BBR via inhibiting mTOR/p70S6K signaling, and imply that it may be possible to develop BBR as a new medication of anti-A β pathology, by which BBR both inhibits ribosylation-induced A β generation and promotes A β clearance, and consequently help to moderate the strong drive to relieve A β pathology.

Abbreviations

AD

Alzheimer's disease; A β : β -amyloid; SP:senile plaques; APP:amyloid precursor protein; NFTs:neurofibrillary tangles; AGEs:advanced glycation end products; mTOR:mammalian target of Rapamycin; MWM:morris water maze; RAGE:advanced glycation end products receptor; CMA:chaperone-mediated autophagy; PD:Parkinson's disease; HD:Huntington's disease; ER β :estrogen receptor β ; ATG:autophagy related protein; BBR:Berberine; N2a:Neuro-2a; DMEM/F12:Dulbecco's Modified Eagle Medium Nutrient Mixture F-12;

FBS:fetal bovine serum; sAPP α :soluble α -fragment; sAPP β :soluble β -fragment; AICD:APP intracellular domain; CNS:central nervous system; LC3:light chain 3; AEP:asparagine endopeptidase; ROS:reactive oxygen species.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Y-Y W and Q Y performed the in vitro and in vivo experiment and drafted the manuscript; Z-T H and Q Z contributed to the behavioral tests and manuscript preparation; J L and M-H Y performed the data acquisition and analyses; Z-Y C and L-Q W contributed to the funding acquisition, study concept and manuscript revision; All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and followed the ethical standards approved by the Research Ethics Committee of Chongqing General Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

BBR promotes autophagy by regulating mTOR signaling pathway in N2a cells. a Western blot analysis the expression of p-mTOR and p-p70S6K in Rapamycin or BBR treated cells. Quantification is presented in b. c Western blot analysis the expression of autophagy related proteins in Rapamycin or BBR treated cells. Quantification is presented in d. e Immunofluorescence detected the expression of p-mTOR and LC3B. The data are shown as mean $\pm$ SEM. * $p < 0.05$. Scale bars=100 μ m. BBR decreases BACE1, γ -secretase and enhances A β -degrading enzymes Neprilysin in N2a cells. We further studied the effect of BBR on A β

associated kinase in N2a cells. As shown in Figure 2a, b, compared with the control group, BBR treatment inhibited the expression of β -secretase, BACE1 and γ -secretase, PS1 and PS2, and enhanced the expression of A β decomposition enzyme, Neprilysin. This effect was similar to Rapamycin. Next, immunofluorescence staining was used to detect the effect of BBR on β -secretase, BACE1 and γ -secretase, PS2. As shown in Figure 2c, BBR treatment decreased the fluorescence intensity of BACE1 and PS2 proteins, which was consistent with the effect of Rapamycin.

Figure 2

BBR regulated the expression of A β associated kinase in N2a cells.a Western blot analysis the expression of BACE1, PS1, PS2 and Neprilysin in Rapamycin or BBR treated cells. Quantification is presented in b. c Immunofluorescence detected the expression of BACE1 and PS2. The data are shown as mean $\pm$ SEM. *p < 0.05. Scale bars=100 μ m. BBR counteracts the activation of mTOR signaling pathway, the inhibition of autophagy, the increase of BACE1, γ -secretase and the decrease of Neprilysin induced by D-ribose in APP/PS1 mice. It has been reported that AGEs can bind with RAGE and then interact with glial cells to promote the aggregation of A β and the hyperphosphorylation of tau protein. D-ribose inducing

ribosylation and the generation of excessive AGEs are an important cause of tau hyperphosphorylation and the formation of NFTs. To clarify the mechanism by which BBR regulating mTOR signaling pathway to promote autophagy, we first analyzed the effect of BBR and D-ribose on mTOR signaling pathway. D-ribose intraperitoneal injecting increased the expression of AGE, p-mTOR and p-p70S6K protein in APP/PS1 mice brain. When mice injected with D-ribose were given BBR intraperitoneally, the increase of AGE, p-mTOR and p-p70S6K protein expression was inhibited, which was similar to the effect of AGE inhibitors (Fig. 3a, b). Then we examined the effect of BBR and D-ribose on autophagy-related proteins. D-ribose intraperitoneal injecting decreased the expression of Beclin1, ATG3 and LC3B, and increased p62 protein in APP/PS1 mice brain. After additional intraperitoneal administration of BBR, the decrease of Beclin1, ATG3 and LC3B, and the increase of p62 protein expression was inhibited, this was consistent with the effect of AGE inhibitors (Fig. 3c, d). Then we investigated the effect of BBR on A β associated kinase in APP/PS1 mice. As shown in Figure 3 e, f, compared with the control group, D-ribose treatment increased the expression of β -secretase, BACE1 and γ -secretase, PS1 and PS2, and decreased the expression of A β decomposition enzyme, Neprilysin. When the D-ribose treated mice were given BBR intraperitoneally, the expression of BACE1, PS1 and PS2 reduced significantly, concurrently, the expression of Neprilysin increased dramatically.

Figure 3

BBR blocked the mTOR signaling pathway, regulated autophagy and A β associated kinase in APP/PS1 mice. a Western blot analysis the expression of AGE, p-mTOR and p-p70S6K in D-ribose group, D-ribose supplementary AGE inhibitors group and D-ribose supplementary BBR group. Quantification is presented in b. c Western blot analysis the expression of autophagy related proteins in D-ribose group, D-ribose supplementary AGE inhibitors group and D-ribose supplementary BBR group. Quantification is presented

in d. e Western blot analysis the expression of BACE1, PS1, PS2 and Neprilysin in D-ribose group and D-ribose supplementary BBR group. Quantification is presented in f. The data are shown as mean $\pm$ SEM. n=10. *p < 0.05. BBR improves spatial learning and memory and inhibits the A β 1-42 expression of APP/PS1 mice. The spatial learning and memory of APP/PS1 mice was performed using the MWM test followed the schematic diagram of experimental process (Fig. 4a). On the 5 days space navigation training, the four experimental groups all showed a time dependent decrease of escape latency. Compared with the D-ribose supplementary AGE inhibitor group and the D-ribose supplementary BBR group, the D-ribose treatment group had longer escape latency from day 2 to day 5 (p $\leq$ 0.05) (Fig. 4b). After removing the platform, the time in the platform location, the times of across platform and the retention time in the target quadrants of D-ribose treatment group had significantly decreased compared with control group, which was abrogated in the D-ribose supplementary AGE inhibitor group and the D-ribose supplementary BBR group (Fig. 4c, d, e). These differences were visualized in the representative mouse trajectories taken by APP/PS1 mice during the space exploration task (Fig. 4f). Immunofluorescence staining was used to intuitively observe the effect of BBR on A β in APP/PS1 brain. Compared with D-ribose group, BBR treated group showed significant decreases in the immunoreactive region of A β 1-42 in APP/PS1 mice hippocampus (Fig. 4g). These results indicated that BBR improved spatial learning and memory, promoted the degradation of A β 1-42 in the hippocampus of APP/PS1 mice.

Figure 4

BBR regulated the spatial learning and memory and the Aβ₁₋₄₂ expression of APP/PS1 mice. a Experiment and timeline procedure. 16 weeks APP/PS mice were divided into 4 groups, control group treated with vehicle, D-ribose group, D-ribose supplementary AGE inhibitor group and D-ribose supplementary BBR group. b Average escape latency of the 5 days space navigation training. The time in the platform location (c), the times of across platform (d), the retention time in the target quadrants (e), and the

representative mouse trajectories (f) were observed during the space exploration task after removing the platform. g Immunofluorescence detected the expression of A β 1-42 in the hippocampus of D-ribose group and D-ribose supplementary BBR group. The data are shown as mean $\pm$ SEM. n=10. *p < 0.05 vs. the escape latency of day 1. #p < 0.05 vs. the D-ribose group. Scale bars=150 μ m.

Figure 5

Schematic diagram of the BBR possible mechanisms.