Berberine Promotes TREM2-Dependent Phagocytosis of Amyloid- β By Microglia

Yang-Yang Wang

Chongqing General Hospital, University of Chinese Academy of Sciences

Zhen-Ting Huang

Chongqing General Hospital, University of Chinese Academy of Sciences

Qian Zou

Chongqing General Hospital, University of Chinese Academy of Sciences

Yin-Shuang Pu

Chongqing General Hospital, University of Chinese Academy of Sciences

Ming-Hao Yuan

Chongqing General Hospital, University of Chinese Academy of Sciences

Feng Jing

Chongqing General Hospital, University of Chinese Academy of Sciences

Sheng-Yuan Wang

Chongqing General Hospital, University of Chinese Academy of Sciences

Zhiyou Cai (✉ caizhiyou@ucas.ac.cn)

Chongqing General Hospital, University of Chinese Academy of Sciences.

Research

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Abstract

Background: The production and accumulation of amyloid- β ($A\beta$) is the most important pathological feature of Alzheimer's Disease (AD), and the deficiency of $A\beta$ clearance contributes to the progression of AD. TREM2-dependent microglial activation may be the key to $A\beta$ clearance. BBR plays the neuroprotective role in the progression of AD by inhibiting $A\beta$ production and promoting $A\beta$ degradation. However, the specific relationship between BBR and microglial activation remains unclear. Thus, we aimed to investigate whether BBR can inhibit the pathological progression of $A\beta$ in AD by changing the phenotype of microglia.

Methods: Western blot and Immunofluorescence staining were applied to detect the effects of BBR on the transformation of resting microglia to different phenotypes. ELISA, Immunohistochemistry and Immunofluorescence were used to detect the effect of BBR on microglial phagocytosis of $A\beta$. Morris water maze (MWM) test was applied to test the effect of BBR on the spatial learning and memory of experimental animals.

Results: Firstly, BBR promoted the phagocytosis of $A\beta_{1-42}$ by BV2 cells. Secondly, BBR promoted the changes of microglia to phenotypes M2 and DAM *in vivo* and *in vitro*, which were in close proximity to $A\beta$ and reduced $A\beta$ aggregation. Finally, BBR ameliorated spatial learning and memory impairment in APP/PS1 mice.

Conclusion: BBR could enhance the phagocytosis of microglia, which decreased $A\beta$ level and improved the spatial learning and memory of APP/PS1 mice.

Background

Alzheimer's disease (AD), the most common progressive neurodegenerative disorder, is characterized by neuronal loss, synaptic dysfunction, and cognitive decline. The major pathological hallmark of AD is the extracellular aggregation and hypometabolism of amyloid- β ($A\beta$) and the intracellular aggregation of hyperphosphorylated tau in the brain [1]. The imbalance between the production and clearance of $A\beta$ leads to increased concentrations of different forms of $A\beta$, which in turn leads to neuronal damage and death and exacerbates the pathological progression of AD [2].

Microglia are the predominant resident immune cells located throughout central nervous system (CNS), which account for 10–15% of all glial cells [3]. Microglia are the first and most important line of immune defense in CNS, which can constantly remove damaged or useless nerves, plaques and infectious substances to maintain the development and homeostasis of CNS. Microglia has been shown to play different roles in the pathogenesis of AD. On the one hand, microglial phagocytosis is one of the most critical mechanisms of $A\beta$ clearance, which can limit amyloid-associated lesions [4]. On the other hand, activated by phagocytosis of $A\beta$, microglia promotes amyloid pathology by releasing inflammatory cytokines [5]. These different effects may be attributable to different microglia phenotypes: neurotoxic pro-inflammatory cells (M1) or neuroprotective anti-inflammatory cells (M2) [6]. The corresponding

biomarkers of classical M1 include iNOS and CD32, which can release a large number of pro-inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α , etc., and damage CNS. The corresponding biomarkers of M2 include Arg1 and CD206, which are capable of anti-inflammatory cytokines such as IL-10 and TGF- β , promoting nerve tissue repair and nerve regeneration [7]. Moreover, a recent single-cell RNA-seq study has revealed a unique microglia type in a mouse model of AD, named "disease-associated microglia" (DAM, marker CD11c), that is actively involved in the breakdown and digestion of amyloid plaques [8].

Triggering receptor expressed on myeloid cells 2 (TREM2) is a member of the TREM family of innate immune receptors that are expressed in microglia, dendritic cells, macrophages, and osteoclasts. TREM2 could bind phospholipids and other polyanionic ligands and transmit intracellular signals through the related adaptor DNAX-activation protein 12 (DAP12), promoting the survival, proliferation, phagocytosis, and secretion of cytokines and chemokines [9, 10]. Previous convincing studies have identified the rare mutation (R47H) in TREM2 as a genetic risk factor for AD [11, 12]. Studies in AD mice (APP/PS1) have found that the TREM2 R47H variant increases the risk of AD by impairing the function of TREM2 and enhancing neurodystrophy around plaques [13]. Another study in 5 $\times$ FAD mice and humans with AD found that TREM2 haplodeficiency may disrupt the formation of neuroprotective microglial barriers that regulate amyloid compaction and insulation [14]. Similarly, in PS2APP AD mouse models, TREM2 deficiency reduced the accumulation of late-stage amyloid plaques, increased the A β 42 to A β 40 ratio, and exacerbated axonal dystrophy and dendritic spine loss [15].

Berberine (BBR) is a natural chemical found in a variety of plants, including *Coptis chinensis*, European barberry, Tree turmeric, Goldenseal, *Phellodendron* and so on [16]. These plants have historically been used to treat a wide range of ailments, including intestinal infections, diarrhea and ulcers. Current research has found that BBR could be used to help treat diabetes [17], high cholesterol [18], obesity [19], cardiovascular disease [20], musculoskeletal disorders [21] and even gynecological cancers [22]. In addition, BBR shows great therapeutic potential in neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases [23].

In AD pathologic progression, BBR not only alleviates A β pathology by inhibiting β / γ secretases, but also weakens neuronal damage caused by A β , and improves glial hyperplasia and cognitive impairment [24–26]. Studies have shown that autophagy is defective in AD and cannot remove cellular garbage normally [27]. However, BBR intervention can alleviate cognitive decline in AD mice by inhibiting A β production, Tau hyperphosphorylation and autophagy clearance [28, 29]. Our previous study found that BBR ameliorates ribosylation induced A β pathology by inhibiting mTOR/p70S6K signaling and promoting autophagy, thereby improving spatial learning and memory in APP/PS1 mice [30].

In this study, we hypothesized that BBR promotes microglial phenotypic changes, thereby regulating TREM2-dependent phagocytosis of A β by microglia. To test this hypothesis, we performed multiple experiments in BV2 cells and transgenic APP/PS1 transgenic AD model mice.

Methods

Drugs, antibodies and animals

Berberine chloride (HY-18258, purity 99.16%) was purchased from MedChemExpress (MCE, NJ, USA), Berberine hydrochloride tablets (100 mg/tablet) were purchased from Sanofi Minsheng Health Pharmaceutical Co. Ltd (Hangzhou, China). Amyloid- β Peptide (1–42) human (P9001) was purchased from Beyotime (Shanghai, China). Antibodies against CD206 (18704-1-AP), CD32 (15625-1-AP) and IL-10 (20850-1-AP) were purchased from Proteintech (IL, USA), β -Amyloid (8243), Iba1 (17198) and DAP12 (12492) were purchased from Cell Signaling Technology (MA, USA), IL-1 β (ab9722) was purchased from abcam (MA, USA), β -Amyloid (sc-28365) was purchased from Santa Cruz Biotechnology (TX, USA), CD11c (117304) was purchased from BioLegend (CA, USA), and TREM2 (bs-2723R) was purchased from Bioss Biotechnology (Beijing, China). Wild-type and APP/PS1 transgenic mice (C57BL/6, male) were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China).

Morris water maze test

MWM is carried out in a circular tank with a diameter of 1.2 m and a depth of 0.4 m, with water temperature maintained at $25 \pm 1^\circ\text{C}$. A circular escape platform (8 cm diameter) was fixed and hidden 1 cm in the middle of the northeast quadrant below the water surface. The adaptive mice were trained 4 times per day for 6 consecutive days as acquisition trials. During the trial, mice were placed into the different quadrants and allowed to swim freely for 60 s to climb up and stay on the platform for 5 s. If a mouse failed to reach the platform within 60 s, it was guided to the platform for an extra 30 s. The time the mice took to reach the platform was considered its escape latency and was recorded along with the swim trajectory by ANY-maze video tracking software (Stoelting Co., USA). For the probe test, mice were placed in the opposite quadrant from the original platform quadrant and tested for 60 s without a platform. The time spent in the four quadrants, the distance to the platform, the distance in the original platform quadrant and the number of platform crossings were observed and recorded.

Cell culture and transfection

BV2 cells were stored by our laboratory and cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS, Invigentech, CA, USA) and 100 U/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin, incubated in humidified atmosphere with 5% CO_2 at 37°C . BV2 cells cultured in six-well plates were transfected with 50 nM TREM2-specific short-interfering RNAs (siRNAs) or siNC (RIBOBIO, Guangzhou, China). The sequences of TREM2-siRNAs were TREM2-siRNA1 (5'-GGAATGGGAGCACAGTCAT-3'), TREM2-siRNA2 (5'-GGCACCAACTTCAGATCCT-3') and TREM2-siRNA3 (5'-CTGTCAACTTCTGCACTTT-3'). To evaluate the effect of BBR on microglia phenotype, BV2 cells were treated with 1 μM BBR and incubated for 24 h.

Cell proliferation and apoptosis assay

Cell Counting Kit 8 (CCK-8, abs50003, Absin, Shanghai, China) was used to assess the proliferation of BV2 cells according to the manufacturer's protocol. In brief, BV2 cells were cultured in 96-well plates with 5.0×10^3 cells/well. After intervention with different reagents, BV2 cells were cultured for 0, 12, 24 or 48 h, followed by adding CCK-8 solution to each well. After further incubation for 4 h, the absorbance of each well was detected at 450 nm using Multiskan FC (Thermo Scientific). Cell apoptosis of BV2 cells was assayed by Hoechst Staining Kit (C0003, Beyotime, Shanghai, China). BV2 cells were seeded on the coverslips in 24-well plates with 1.0×10^5 cells/well. Different treatment groups of BV2 cells in plates were fixed for 10 min and stained with 100 μ l Hoechst 33258 solution per well. The coverslips were installed onto glass slides using Antifade Mounting Medium (Beyotime) and detected by a fluorescence microscope (NEXCOPE).

ELISA analysis

BV2 cells cultured in 12-well plates were treated with A β Peptide (1–42) (Beyotime) at 10 μ M for 24 h. The supernatant of BV2 cells was obtained by centrifugation at 2000 rpm for 20 min, and the cell contents were destroyed by repeated freeze-thaw and release of intracellular components, and then obtained by centrifugation at 2000 rpm for 20 min. A β_{1-42} in cell supernatant and contents was detected using ELISA kits from Jiangsu Meibiao Biological Technology (China) in accordance with the manufacturers' protocols.

Western blot analysis

BV2 cells or brain tissue of APP/PS1 mice were lysed with RIPA Lysis Buffer containing protease inhibitor PMSF (Beyotime), and the total protein extracted was detected by BCA Protein Assay Kit (Beyotime). Equivalent amount of protein were separated on the polypropylene gels with corresponding concentration and transferred to PVDF membranes (Bio-Rad, Hercules, CA, USA). Blocked membranes were incubated with indicated primary antibodies overnight at 4°C. Membranes were then incubated with corresponding secondary antibodies for 1 h at room temperature. Protein bands were visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore Corporation, MA, USA) and Tanon-5200 multi chemiluminescence analysis system (Tanon, Shanghai, China).

Histology and Immunohistochemical staining

After the behavioral test, the brains of APP/PS1 mice were removed and fixed with 4% paraformaldehyde for 48 h. Paraffin sections with a thickness of 4 μ m were cut along the long axis of the sagittal plane by a Rotary Microtome (HM 340E, Thermo Scientific). After deparaffinization and rehydration, antigen-retrieval was performed by microwave oven with citric acid buffer. Then paraffin sections were blocked with 10% goat serum and incubated with indicated primary antibodies overnight at 4°C. After incubation with the secondary antibodies, sections were detected by DAB staining kit, counterstained with hematoxylin, and the target images were observed and captured with a bright field NEXCOPE microscope (NE900, USA).

Immunofluorescence staining

BV2 cells cultured on glass coverslips were washed with PBS and fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. 4 μ m paraffin sections or BV2 cells were blocked with 5% goat serum albumin, and incubated with indicated primary antibodies overnight at 4°C. After incubation with secondary fluorescent antibodies Alexa Fluor® 488 or Alexa Fluor® 594 antibodies (ZSGB-BIO, Beijing, China) for 1 h in the dark, the nucleuses were stained with DAPI (Beyotime). The coverslips were mounted onto glass slides using Antifade Mounting Medium (Beyotime), and the imaging was performed using NEXCOPE microscope (NE900, USA).

Statistical analyses

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

Results

BBR promoted phagocytosis of $A\beta_{1-42}$ by BV-2 cells

Western blot analysis revealed that BBR treatment could significantly increase the protein expression level of TREM2 compared with the control group in BV2 cells (Fig. 1a). To investigate the pathophysiological function of TREM2, siRNA interference was used to knock down TREM2. Western blot results showed that the expression of TREM2 in three different siRNA-targeted interferences was significantly lower than that in the blank control group (Fig. 1b). Based on this result, siTREM2-1 was selected for following knockdown experiments. Then, BV2 cells were stained with Hoechst 33258, a special fluorescent dye that can distinguish apoptotic cells from normal cells, under a fluorescence microscope, and the cell morphology was observed. As shown in Fig. 1c, after BBR treatment, the nuclear morphology did not change, showing diffuse homogeneous blue fluorescence as in normal nuclei. When treated with si-TREM2 or $A\beta_{1-42}$, the cells show apoptotic cell-like nucleus fragmentation, chromosome condensation and strong blue fluorescence. However, the addition of BBR in combination with si-TREM2 or $A\beta_{1-42}$ treatment can restore the nuclear state of apoptosis. The effect of TREM2 on BV2 cell viability was assessed using the CCK8 assay. As shown in Fig. 1d, the cell viability of BBR treatment group decreased slightly by 11.8%, whereas si-TREM2 and $A\beta_{1-42}$ treatment significantly decreased by 53.8% and 62.4%. Compared with si-TREM2 group and $A\beta_{1-42}$ group, BBR supplementation reversed cell viability by 58.1% and 94.3%. These results indicated that BBR has no significant effect on cell viability, and can rescue the decreased cell viability caused by si-TREM2 and $A\beta_{1-42}$. ELISA assay was used to detect the effect of BBR on the $A\beta$ phagocytosis of BV2 cells. In BV2 cell contents, $A\beta_{1-42}$ treatment significantly increased $A\beta_{1-42}$ content compared with the control group, while the addition of BBR significantly reversed this effect (Fig. 1e Left). Similarly, in the supernatant of BV2 cells, $A\beta_{1-42}$ treatment significantly increased $A\beta_{1-42}$ content, while BBR significantly reversed this result (Fig. 1e Right). These data suggest that BBR promotes $A\beta$ phagocytosis in BV2 cells.

BBR promotes the phenotype changes of microglia in vitro.

Microglia cells have different phenotypes. Activated microglia cells include neuro-damaging M1, neuro-protective M2, and a new type of DAM that can significantly limit the progression of AD. Therefore, the role of microglia cells in AD is a double-edged sword. We should take advantage of its favorable side and inhibit its harmful side to achieve the effect of treating AD. In this study, we found that BBR inhibits the transition of resting BV2 cells to M1 and promotes the transition of resting BV2 cells to M2 by regulating TREM2 (Fig. 2a). As shown in Fig. 2b-c, compared with the control group, BBR significantly decreased the expression of M1 markers CD32 and IL-1 β , and significantly increased the expression of M2 markers CD206 and IL-10, while si-TREM2 significantly promoted the expression of CD32 and IL-1 β , and significantly inhibited the expression of CD206 and IL-10. However, compared with si-TREM2 group, BBR supplementation significantly decreased the expression of CD32 and IL-1 β , and significantly increased CD206 and IL-10 expression. In addition, we evaluated the expression of CD32 (Fig. 2d) and CD206 (Fig. 2e) in microglia (IBA1) using immunofluorescence staining, which was consistent with Western blot results. In short, our results prove that BBR could inhibit the transformation of resting microglia to M1 and promote the transformation of resting microglia to M2. Then, immunofluorescence was used to detect BBR's effect on microglial transformation to DAM cells. As shown in Fig. 3a-b, BBR significantly promoted DAM marker CD11c expression and si-TREM2 significantly promoted CD11c expression compared with the control group in microglia (IBA1). However, BBR combined with si-TREM2 treatment did not restore CD11c expression compared with si-TREM2 group, suggesting that BBR may promote microglial conversion to DAM through TREM2. In conclusion, these data demonstrate that BBR promotes BV2 cells transformation to neuroprotective M2 and DAM and inhibit BV2 cells transformation to neurotoxic M1.

BBR promotes the phenotype changes of microglia in vivo.

To further confirm the effect of BBR on microglia phenotypic changes, wild-type (WT) mice, AD control mice (Saline treatment) and AD experimental mice (BBR treatment) were used as animal models. *In vivo* experiments indicated that BBR inhibits the transition of resting microglia to M1 and promotes the transition of resting microglia to M2 and DAM, which was in accordance with *in vitro* results (Fig. 4a). Western blot assay showed that CD32 and IL-1 β expression in the Saline group was significantly higher than that in the WT group. Compared with the Saline group, the expression of CD32 and IL-1 β in AD mice after BBR treatment was significantly decreased, while the expression of CD206 and IL-10 was significantly increased (Fig. 4b-c). Immunofluorescence results showed that the CD11c fluorescence intensity in microglia (IBA1) of AD mice treated with BBR was significantly increased compared with that of Saline group (Fig. 4d). These results suggest that BBR can not only inhibit the transformation of microglia into neurotoxic M1, but also promote the transformation of microglia into neuroprotective M2 and DAM in mice.

BBR promotes phenotypic altered microglia to surround A β in vivo.

The results of both *in vivo* and *in vitro* experiments showed that BBR could promote the transformation of microglia to M2 and DAM phenotypes. Next, we studied the effects of M2 and DAM phenotypic microglia on A β . Here we report that BBR promotes microglial transformation of M2 and DAM to encircle A β and inhibit A β expression through TREM2 (Fig. 5a). Immunofluorescence results showed that BBR promoted the increase of M2 (CD206) in microglia (IBA1), and the increased M2 tended to surround A β (Fig. 5b). Similarly, BBR promoted the significantly increased DAM (CD11c) in microglia (IBA1) to be in proximity to A β (Fig. 5c), which may have the phagocytic effect on A β . Both M2 with anti-inflammatory effect and DAM with phagocytic effect were close to A β , which may have a positive promoting effect on A β clearance. Immunohistochemical results showed that BBR significantly increased the expression of TREM2 and significantly reduced A β plaques compared with AD control group (Fig. 5d), suggesting that BBR may promote the transition of resting microglia to M2 and DAM by promoting the expression of TREM2, thereby leading A β clearance.

BBR regulated the spatial learning and memory of APP/PS1 mice.

The MWM test was performed to evaluate spatial learning and memory of APP/PS1 mice followed the mouse experiment process diagram (Fig. 6a). The escape latency of WT, APP/PS1 control (Saline), and APP/PS1 + BBR groups decreased in a time-dependent way during 5 consecutive days of pre-training. On day 5, APP/PS1 control group had significantly higher escape latency than WT group, while APP/PS1 + BBR had significantly lower escape latency than APP/PS1 control group (Fig. 6b). After pre-training, the platform was removed to measure the platform location time, the across platform times and the retention time in the target quadrants to test the long-term spatial memory ability of experimental mice. As shown in Fig. 6c-e, APP/PS1 control group showed worse spatial memory ability than WT group, while the platform location time, the across platform times and the retention time of APP/PS1 + BBR group were significantly improved compared with that of APP/PS1 control group. Typical trajectories of the mice clearly showed differences in the spatial memory abilities of the three groups (Fig. 6f). These results indicated that BBR could significantly improve spatial learning and memory of APP/PS1 mice.

Discussion

AD is one of the most common diseases leading to cognitive dysfunction in middle-aged and elderly people. The pathogenesis and treatment of AD have always been a problem in the world. Although an endless stream of drugs and treatments have failed to achieve ideal results, the therapies and drugs that target A β have always been the key point in the treatment of AD. Microglia, a type of glial cells, is the first and most important line of immune defense in CNS and plays an important role in the damage and repair of CNS. Activated microglia, including pro-inflammatory M1, anti-inflammatory M2 and phagocytes associated with AD (DAM) could be directly or indirectly involved in the occurrence and development of AD [31, 32]. Microglia is a double-edged sword in AD. On the one hand, A β stimulates and activates microglia to produce inflammatory factors and neurotoxins, leading to neuronal damage and even death

and triggering AD. On the other hand, microglia can protect CNS by phagocytosis of A β [33]. In current study, we found that BBR inhibits the transition of resting microglia to M1 and promotes the transition of resting microglia to M2 and DAM, which may play a positive role in inhibiting the pathological progression of A β .

In recent years, researchers have turned their attention to the central role of myeloid cells in a variety of pathologies, and TREM2 has been identified as the primary pathology-induced immune signaling center. They observed changes in the levels of TREM2 in various contexts of neurodegenerative change, further underlining the importance of TREM2 in neurological disorders and the exciting possibilities for treatment targeting TREM2, particularly in AD [34]. Colonna et al had previously shown that microglia surround A β plaques, preventing the damaged area from growing [10], while microglia in mice lacking TREM2 allow these plaques to spread and damage neurons more widely [35]. Research found that TREM2 deficiency impairs A β degradation *in vitro* and *in vivo*, which demonstrates TREM2 as a microglial A β receptor transducing physiological and AD-related pathological effects associated with A β [36]. In addition, TREM2 is a receptor required to activate DAM and is critical for DAM to phagocytosis of A β and improves AD pathology, which may have important significance for the future treatment of neurodegenerative diseases such as AD [8]. In this study, we report that BBR promotes the transition of resting microglia to M2 and DAM in a TREM2-dependent way.

BBR is a natural drug extracted from plants such as *Coptis chinensis* and has a broad spectrum of anti-inflammatory and anti-tumor activities. The pharmacological role of BBR in neurodegenerative diseases is gradually being reported. For example, BBR could improve brain dopa/dopamine levels to ameliorate Parkinson's Disease by regulating gut microbiota [37]. In Huntington's Disease transgenic mice, BBR could enhance the autophagy function to promote the degradation of mutant Huntington protein, and effectively alleviate the motor dysfunction of model mice, prolong their survival time [38]. A systematic review of pre-clinical studies [39] shows the neuroprotective effects of BBR in AD animal models: BBR showed significant memory-improving activity through a variety of mechanisms including anti-inflammatory, anti-oxidative stress, cholinesterase inhibition and anti-amyloid. In this study we showed that BBR promotes the enveloping and phagocytosis of A β by regulating microglia phenotypic changes, and ultimately reduces A β content and promotes the improvement of cognitive impairment in AD mice.

However, the main limitation of this study is that only A β pathology was studied. AD is a complex disease driven by multiple factors, and many therapeutic strategies based on reducing A β have failed in clinical trials. This suggests that the treatment of AD should not be based on a single cause but on a number of different pathways. Given the complexity of AD pathology and the multiple efficacy of BBR, we should further investigate the value of BBR in the treatment of AD from different approaches.

Conclusion

In conclusion, we demonstrated that BBR promotes microglial phagocytosis of A β in BV2 cells and APP/PS1 mice (Fig. 7). First, BBR promotes the phagocytosis of A β in BV2 cells by increasing the

expression of TREM2. Second, BBR inhibits the transition of resting microglia to M1 and promotes the transition of resting microglia to M2 and DAM *in vivo* and *in vitro*. Thirdly, BBR promotes the increase of M2 and DAM towards A β and decreases the content of A β by increasing the expression of TREM2. Moreover, BBR improves the spatial learning and memory of APP/PS1 mice. Taken together, these findings reveal novel molecular pathways through which BBR improves A β pathology and promotes A β reduction by altering microglial phenotypes. It is suggested that BBR may be developed as a novel agent for the treatment of AD by altering microglia phenotype to promote A β clearance, thus contributing to the alleviation of A β pathology.

Abbreviations

A β : amyloid- β ; AD: Alzheimer's Disease; BBR: Berberine; CCK8: Cell Counting Kit 8; CNS: Central nervous system; DAP12: DNAX-activation protein 12; DMEM: Dulbecco's Modified Eagle Medium; FBS: Fetal bovine serum; MWM: Morris water maze; siRNAs: Short-interfering RNAs; TREM2: Triggering receptor expressed on myeloid cells 2; WT: wild-type

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Y-Y W performed the *in vitro* and *in vivo* experiment and drafted the manuscript; Z-T H and Q Z contributed to the behavioral tests and western blot analysis; Y-S P and M-H Y performed Immunohistochemical and Immunofluorescence staining; F J performed ELISA and cell experiments; S-Y W performed the data acquisition and analyses; Z-Y C 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 data 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

All authors declare that they have no conflict of interest.

Author details

¹Chongqing Key Laboratory of Neurodegenerative Diseases, Chongqing, 400013, China. ²Department of Neurology, Chongqing General Hospital, University of Chinese Academy of Sciences, Chongqing, 400013, China.

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Figures

Figure 1

BBR promoted phagocytosis of Aβ₁₋₄₂ by BV-2 cells. Western blot was used to detect the expression level of TREM2 in BV-2 cells after BBR treatment (0.5/1 μM, 24 h) (a) and si-TREM2s, Mock or NC transfection (b). (c) Hoechst staining of apoptotic BV2 cells after BBR (0.5/1 μM, 24 h), si-TREM2, Aβ₁₋₄₂ (10 μM, 24 h) treatment. Scale bar = 10 μm. (d) CCK8 assay was used to detect the effect of BBR (1 μM, 24 h), siRNA

and Aβ1-42 (10 μM, 24 h) on BV-2 cells activity. (e) ELISA was used to measure the phagocytosis of Aβ1-42 by BV-2 cells. (*p < 0.05, **p < 0.01 and ***p < 0.001)

Figure 2

BBR promotes the M1/M2 phenotype changes of BV2 cells. (a) Schematic diagram depicting the mode of BBR regulating the phenotype of BV2 cells. BV2 cells were treated with BBR (1 μM, 24 h), transfected with si-TREM2 or their combination. (b) The CD32, IL-1β (M1 marker) and CD206, IL-10 (M2 marker) protein

level was tested by Western blot. (c) CD32/CD206/IL-1 β /IL-10: GAPDH ratios. Immunofluorescence was used to detect the fluorescence intensity of CD32 (d, green) and CD206 (e, green). In addition, nuclei (DAPI, blue) and microglia (IBA1, red) was staining. (*p < 0.05, **p < 0.01 and ***p < 0.001 versus Control group, ##p < 0.01 and ###p < 0.001 versus BBR group). Scale bar = 50 μ m.

Figure 3

BBR promotes the DAM phenotype changes of BV2 cells. (a) Schematic diagram depicting the mode of BBR regulating the phenotype of BV2 cells. BV2 cells were treated with BBR (1 μ M, 24 h), transfected with si-TREM2 or their combination. (b) Immunofluorescence was used to detect the fluorescence intensity of CD11c (DAM marker, green). In addition, nuclei (DAPI, blue) and microglia (IBA1, red) was staining. Scale bar = 50 μ m.

Figure 4

BBR promotes the phenotype changes of microglia. (a) Schematic diagram depicting the mode of BBR regulating the phenotype of microglia cells. The experimental mice were divided into WT control group, AD control group (Saline treatment, 100 mg/kg/d), and AD experimental group (BBR treatment, 100 mg/kg/d). (b) The CD32, IL-1 β (M1 marker) and CD206, IL-10 (M2 marker) protein level was tested by Western blot. (c) CD32/CD206/IL-1 β /IL-10: GAPDH ratios. (d) Immunofluorescence was used to detect

the fluorescence intensity of CD11c (green) in the cortex of WT and AD mice. In addition, nuclei (DAPI, blue) and microglia (IBA1, red) was staining. Scale bar = 50 μ m. (**p < 0.01 and ***p < 0.001 versus WT group, #p < 0.05 and ##p < 0.01 versus Saline group).

Figure 5

BBR promotes phenotypic altered microglia to surround Aβ. (a) Schematic diagram depicting the mode of BBR regulating the phenotype of microglia cells and Aβ. (b) Immunofluorescence was used to detect the

fluorescence intensity of CD206 (red) in the cortex of WT and AD mice. DAPI (blue), A β (green). (c) Immunofluorescence was used to detect the fluorescence intensity of CD11c (green) in the cortex of WT and AD mice. DAPI (blue), A β (red). Scale bar = 50 μ m. (d) TREM2 and A β detection was performed by immunohistochemistry in the cortex of WT and AD mice. Scale bar = 50 μ m.

Figure 6

BBR regulated the spatial learning and memory of APP/PS1 mice. (a) Experiment and timeline procedure. 16 weeks mice were divided into 3 groups, WT group, APP/PS1+Saline group and APP/PS1+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. (**p < 0.01 and ***p < 0.001 versus WT group, ##p < 0.01 and ###p < 0.001 versus Saline group).

Figure 7

Schematic diagram of possible mechanisms of BBR regulating microglia. BBR could inhibit the transformation of resting microglia to pro-inflammatory M1 and promote the transformation of resting microglia to anti-inflammatory M2. In addition, BBR may also promote the transformation of M1 to M2. At the same time, BBR could promote the transformation of microglia to DAM by promoting the expression of TREM2, and DAM can surround Aβ and then decrease Aβ to protect neurons.