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•Original article•

Bear bile powder alleviates Parkinson's disease-like behavior in mice by inhibiting astrocyte-mediated neuroinflammation

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[ABSTRACT] Parkinson's disease (PD) is a common neurodegenerative disease in middle-aged and elderly people. In particular, increasing evidence has showed that astrocyte-mediated neuroinflammation is involved in the pathogenesis of PD. As a precious traditional Chinese medicine, bear bile powder (BBP) has a long history of use in clinical practice. It has numerous activities, such as clearing heat, calming the liver wind and anti-inflammation, and also exhibits good therapeutic effect on convulsive epilepsy. However, whether BBP can prevent the development of PD has not been elucidated. Hence, this study was designed to explore the effect and mechanism of BBP on suppressing astrocyte-mediated neuroinflammation in a mouse model of PD. PD-like behavior was induced in the mice by intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (30 mg·kg⁻¹) for five days, followed by BBP (50, 100, and 200 mg·kg⁻¹) treatment daily for ten days. LPS stimulated rat C6 astrocytic cells were used as a cell model of neuroinflammation. The results indicated that BBP treatment significantly ameliorated dyskinesia, increased the levels of tyrosine hydroxylase (TH) and inhibited astrocyte hyperactivation in the substantia nigra (SN) of PD mice. Furthermore, BBP decreased the protein levels of glial fibrillary acidic protein (GFAP), cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS), and up-regulated the protein levels of takeda G protein-coupled receptor 5 (TGR5) in the SN. Moreover, BBP significantly activated TGR5 in a dose-dependent manner, and decreased the protein levels of GFAP, iNOS and COX2, as well as the mRNA levels of GFAP, iNOS, COX2, interleukin (IL)-1 β , IL-6 and tumor necrosis factor- α (TNF- α) in LPS-stimulated C6 cells. Notably, BBP suppressed the phosphorylation of protein kinase B (AKT), inhibitor of NF- κ B (I κ B α) and nuclear factor- κ B (NF- κ B) proteins *in vivo* and *in vitro*. We also observed that TGR5 inhibitor triamterene attenuated the anti-neuroinflammatory effect of BBP on LPS-stimulated C6 cells. Taken together, BBP alleviates the progression of PD mice by suppressing astrocyte-mediated inflammation *via* TGR5.

[KEY WORDS] Parkinson's disease; Bear bile powder; Astrocyte; Neuroinflammation; Takeda G protein-coupled receptor 5

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Introduction

Parkinson's disease (PD) has become the second most common neurodegenerative disease after Alzheimer's disease (AD) [1]. The pathogenesis of PD is thought to result from the degeneration and necrosis of dopaminergic neurons in the substantia nigra (SN) and the significant reduction of dopamine in the striatum, accompanied by bradykinesia,

myotonia, static tremor and postural reflex disorder [2, 3]. The prevalence of PD is 2%–3% in the elderly over 65 years old, and increases with age, posing a heavy burden on the families and society [4]. Nowadays, the pathogenesis of PD is still unclear, and the treatment regimens are limited, which improve symptoms, but can not prevent the development of the disease, let alone cure it [5]. Therefore, it is urgent to search for new safe and effective therapeutic agents.

Neuroinflammation has been implicated to play an important role in PD pathogenesis [6]. Studies showed that both PD patients and PD animal models induced by overexpression of α -synuclein (α -syn), 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) manifested excessive gliosis and significant inflammatory responses in the brain [7-11]. Previously, microglia was con-

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sidered as the primary inflammatory cells in the central nervous system (CNS). In contrast, the inflammatory activation of astrocytes is generally more persistent and thought to be more important in the neuroinflammation of PD [12]. Over-activation of astrocytes reduced the secretion of neurotrophic factors and promoted the secretion of pro-inflammatory cytokines, such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor- α (TNF- α), leading to the damage of dopaminergic neurons [13]. Many treatments have been reported to attenuate PD progression by targeting the suppression of astrocyte activation. For instance, caffeic acid alleviated dopaminergic neuron damage in PD mice by inhibiting the release of inflammatory cytokines in astrocytes [14]. Dexmedetomidine (DEX) suppressed astrocyte activation *via* the AMPK/NF- κ B pathway and improved dyskinesia in PD mice [15]. Glucagon like peptide-1 receptor agonist effectively prevented abnormal astrocyte lesions and relieved the symptoms of PD model mice [16]. These findings indicate that neuroinflammation mediated by astrocytes is indeed involved in the development of PD.

Bile acids play anti-inflammatory effect and perform immune and nutrient metabolism regulatory roles mainly through binding to bile acid membrane receptor (takeda G protein-coupled receptor 5, TGR5) and nuclear receptor (farnesoid X receptor, FXR) [17]. FXR is mainly expressed in the liver, intestine, kidneys and central neurons, and involved in the synthesis of bile acids, which regulates the metabolism of glycolipids by controlling the enterohepatic circulation of bile acids [18]. TGR5 is distributed in a variety of tissues and cells, including the liver, ileum, colon, central neurons, microglia and astrocytes [19, 20]. TGR5 has various cellular biological functions after activation, such as regulating energy balance, improving insulin resistance, anti-atherosclerosis, and anti-inflammation [21, 22]. TGR5 activation promoted cAMP generation and inhibited NF- κ B activation *via* the cAMP-PKA pathway [23]. In addition, it reduced the expression of chemokines in macrophages *via* the AKT-mTOR signaling pathway, thus inhibiting inflammatory reaction and improving insulin resistance [24]. TGR5 agonist inhibited myeloid cell activation and attenuated experimental autoimmune encephalomyelitis in mice [25]. Therefore, TGR5 may be a proper target for the treatment of inflammation and inflammatory-related diseases.

Bear bile powder (BBP) is a dry powder collected from bear gall bladder bile, which has multiple pharmacological activities, such as anti-inflammatory, antispasmodic, tumor suppressive, lipid regulatory and microcirculation promotive effects [26-28]. The main components of BBP are taurochenodeoxycholic acid (TCDCA) and tauroursodeoxycholic acid (TUDCA), both of which can significantly activate TGR5 [29, 30]. TCDCA played a vital role in cell apoptosis, immune response and inflammation by activating TGR5 [29]. In contrast, TUDCA ameliorated LPS-induced cognitive dysfunction in mice by inhibiting the NF- κ B pathway [31]. In addition, BBP alleviated liver injury by reducing liver inflammation and at-

tenuated febrile epilepsy by regulating neurotransmitters and inhibiting neuroinflammation [32, 33]. Recent research also showed that BBP exerts anti-neuroinflammatory effect by activating TGR5 both *in vivo* and *in vitro* [34]. However, it is still unknown whether BBP can affect the activation of astrocytes *via* TGR5 and thus prevent the development of PD. In the present study, we investigated the protective effect of BBP on PD mice and explored its mechanism from the perspective of inhibiting astrocyte-mediated neuroinflammation. This study may provide evidence for the clinical application of BBP in PD.

Materials and Methods

Reagents

BBP (Cat No. Z10980057) was purchased from Heilongjiang Heibao Pharmaceutical Co., Ltd. (Haerbin, China), which mainly contains six bile acid components: TCDCA (23.3%), TUDCA (23.1%), ursodeoxycholic acid (UDCA, 0.2%), taurohyodeoxycholic acid (THDCA, 11.1%), hyodeoxycholic acid (HDCA, 1.4%), and taurocholic acid (TCA, 1.2%). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, Cat No. MB3783), lipopolysaccharide (LPS, Cat No. MB5198), minocycline (MC, Cat No. MB1477), triamterene (Cat No. MB2034) and dexamethasone (DEX) (Cat No. MB1434) were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Antibodies against TH (Cat No. Ab75875), IBA1 (Cat No. Ab178846), GFAP (Cat No. Ab4648), iNOS (Cat No. ab15323) and TGR5 (Cat No. ab72608) were bought from Abcam (CB, UK). Antibodies against β -actin (Cat No. 12413), GAPDH (Cat No. 5174), COX2 (Cat No. 1282S), AKT (Cat No. 4685S), p-AKT (Cat No. 9271S), I κ B α (Cat No. 44D4), p-I κ B α (Cat No. 2859S), NF- κ B (Cat No. 8242S), and p-NF- κ B (Cat No. 3033L) were bought from Cell Signal Technology (MA, USA). PCR primers including GAPDH, GFAP, COX2, iNOS, IL-1 β , IL-6 and TNF- α were provided by Shanghai Jierui Biological Engineering Co., Ltd. (Shanghai, China).

Animals and drug treatment

Male C57BL/6 mice, 10–12 weeks old and 28–30 g in weight, were obtained from the Animal Experimental Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China). All the mice were kept under 40%–60% humidity in a 12 h dark/light cycle at 25 \pm 2 $^{\circ}$ C. After acclimation for two weeks, the mice were randomly divided into six groups according to the experimental requirements: a control group, a MPTP group, a positive drug group (minocycline, 50 mg \cdot kg $^{-1}$) and MPTP + BBP (50, 100, and 200 mg \cdot kg $^{-1}$) groups. The control group was intraperitoneally injected with normal saline, while the other groups were intraperitoneally injected with MPTP (30 mg \cdot kg $^{-1}$) for five consecutive days to establish a subacute PD mouse model [2]. On day 6, the mice in the treatment groups were given minocycline and BBP (50, 100, and 200 mg \cdot kg $^{-1}$) daily, while the other mice received normal saline. Behavioral tests including the pole test and the open field test were performed after 10 days of administration. At the end of the experiments, the mice

were anesthetized and sacrificed to obtain the brain tissues including the substantia nigra for further analysis. All the drugs were dissolved with normal saline. The animal experiments were conducted according to a protocol approved by the University Animal Care and Use Committee of SHUTCM (Ethics number: PZSHUTCM2212050002).

Behavioral tests

The motor ability of mice was evaluated by the open field test [35]. First, the mice were kept in the experimental environment to adapt for 1–2 h. Then, the mice were individually placed in a central area at the bottom of an open field (50 cm × 50 cm × 50 cm). The motion track of the mice was continuously collected by a video tracking system. Their motor ability was evaluated based on the total movement distance within 5 min.

Motor impairment in mice was assessed by the pole test [36]. Briefly, a pole with a diameter of 1 cm and a length of 50 cm was fixed to the ground, and a small ball was installed on the top of the pole. Both the pole and the ball were covered with a gauze to prevent the mice from slipping. Before the experiment, all the mice were trained to climb the pole. After adaptive training, the mice were placed on the ball and the total time spent by each mouse from the ball to the base of the pole was recorded. Each mouse was tested for three times with an interval of more than 1 h.

Cell culture and treatment

Rat C6 astrocytic cells were provided by the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) was used to culture the cells at 37 °C with 5% CO₂. C6 cells were starved with serum-free DMEM for 24 h and seeded in a 96-well culture plate at a density of 1.5×10^6 cells/mL. Next day, the cells were first treated with BBP (25, 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$) and DEX (1 $\mu\text{mol}\cdot\text{L}^{-1}$) or BBP (100 $\mu\text{g}\cdot\text{mL}^{-1}$), BBP (100 $\mu\text{g}\cdot\text{mL}^{-1}$) + triamterene (20 $\mu\text{mol}\cdot\text{L}^{-1}$) and triamterene (20 $\mu\text{mol}\cdot\text{L}^{-1}$) for 2 h, followed by induction with 1 $\mu\text{g}\cdot\text{mL}^{-1}$ LPS for 24 h. The nitric oxide (NO) release was detected by Greiss reagent as previously described [34]. Cell viability was detected by CCK-8 assay.

Immunocytochemistry (ICC)

C6 cells were treated with BBP for 2 h and then induced with 1 $\mu\text{g}\cdot\text{mL}^{-1}$ LPS for 6 h. The cells were fixed with 4% paraformaldehyde, penetrated with PBS containing 0.3% Triton X-100, blocked in 5% donkey serum and incubated with NF- κ B primary antibody (1 : 300) at 4 °C. Next day, the fluorescent secondary antibody conjugated with Alexa-594 (Invitrogen, cat: A32754) was used to incubate the cells. The nuclei were stained with DAPI. Photographs were taken by a fluorescence microscope.

Real-time PCR

The total RNA of C6 cells was extracted by Trizol method. RNA concentrations were determined by Nano Drop. The resultant RNA was reversely transcribed into cDNA on PCR instrument. Taqman SYBR kit (Q511-02, Vazyme, China)

was used to perform real-time PCR, and the obtained mRNA expression was quantified by the delta-delta Ct approach. The primer sequences used are listed in Table 1.

Table 1 Sequences of primers for PCR

Gene	Sequence 5'-3'
<i>Rat-COX2</i>	F: TTCAACACACTCTATCACTGGC R: AGAAGCGTTTGCGGTACTCAT
<i>Rat-GAPDH</i>	F: TGTGAACGGATTGGCCGTA R: TGAAGTGGCGTGGGTAGAG
<i>Rat-IL-1β</i>	F: CACCTCTCAAGCAGAGCACAG R: GGGTCCATGGTGAAGTCAAC
<i>Rat-GFAP</i>	F: TGGCCACCAGTAACATGCAA R: ACTCAAGGTCGAGGTCAAG
<i>Rat-IL-6</i>	F: TAGTCCTTCCTACCCAACTTCC R: TTGGTCCTTAGCCACTCCTTC
<i>Rat-iNOS</i>	F: AGCATCACCCCTGTGTCCACCC R: TGGGGCAGTCTCCATTGCCA
<i>Rat-TNF-α</i>	F: CTGAACTTCGGGGTGATCGG R: GGCTTGCTACTCGAGTTTGAA

TGR5 luciferase assay

HEK293T cells were seeded in 96-well culture plates. After overnight culture, the cells were co-transfected with pCRE-luc, pCMV-TGR5 and Renilla plasmids as previously described [37]. One day after transfection, the cells were exposed to BBP (25, 50 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$) and INT-777 (0.5 $\mu\text{mol}\cdot\text{L}^{-1}$), according to the experimental requirements. After treatment for 5 h, TGR5-luciferase activity was measured using Dual Luciferase Reporter Gene Assay kit (Meilun, Dalian, China) by a multifunctional microplate reader.

Western blot analysis

RIPA lysis buffer containing phosphatase and protease inhibitors was used for extracting the total protein of mouse SN tissues and C6 cells. Then, the supernatant was used for the determination of protein concentrations by BCA method. The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto PVDF membrane. To inhibit nonspecific binding, 5% skim milk was used to block the membrane. Then, the target bands were incubated with primary antibodies against TH (1 : 1000), iNOS (1 : 1000), GFAP (1 : 3000), COX2 (1 : 1000), AKT (1 : 1000), p-AKT (1 : 1000), I κ B α (1 : 1000), p-I κ B α (1 : 1000), NF- κ B (1 : 1000), p-NF- κ B (1 : 1000), β -actin (1 : 5000) and TGR5 (1 : 1000) at 4 °C overnight. Next day, the secondary antibodies of the corresponding species were used to incubate the target bands at room temperature. The ECL luminescent solution was used to visualize the target proteins. Moreover, the gel imaging system was applied to take photographs, and the gray intensity was calculated by Image Pro Plus 6.0.

Immunohistochemistry (IHC)

After anesthesia, the mice were perfused with normal saline and 4% PFA. Mouse brain was removed, fixed with 4% PFA for 24 h, and dehydrated with 10% and 30% sucrose solution for 24 h, before embedded with OCT. Coronal brain sections at 20 μm thickness were obtained and washed with PBS. After penetrated with 0.3% Triton X-100 for half an hour, the sections were blocked with 5% donkey serum for 1 h and incubated with the mixture of GFAP and TH primary antibodies at 4 °C. Next day, the sections were incubated with fluorescent secondary antibody conjugated with Alexa 488 (Invitrogen, cat: A21208) at room temperature for 1 h. After incubation, the nuclei were stained with DAPI and the sections were sealed. The fluorescent images were obtained by an inverted fluorescence microscope.

The target region of SN was defined according to the brain map of mice. Three non-overlapping fields were randomly selected for cell counting under the microscope at $\times 200$ magnification, where positive cells outside the focal plane were not included in the count. The average number of

positive cells in the three fields was regarded as the number of positive cells in the sample [38]. All the slides were scanned and analyzed using an inverted fluorescent microscope (Olympus IX 81, Japan). The number of positive cells for each sample was calculated by Image Pro Plus 6.0 and analyzed by the normalization method (fold/control).

Statistical analysis

Data were analyzed by GraphPad Prism 7 software and are expressed as mean \pm SEM. The One-way ANOVA with Dunnett's post hoc test was used to compare the differences among multiple groups. $P < 0.05$ was considered statistically significant.

Results

BBP improves motor deficits and dopaminergic neurodegeneration in MPTP-induced mice

To evaluate the neuroprotective effect of BBP on MPTP-induced mice, BBP treatment was started five days after MPTP induction. As shown in Figs. 1A–1E, MPTP-induced mice spent much more time to climb down the pole in the

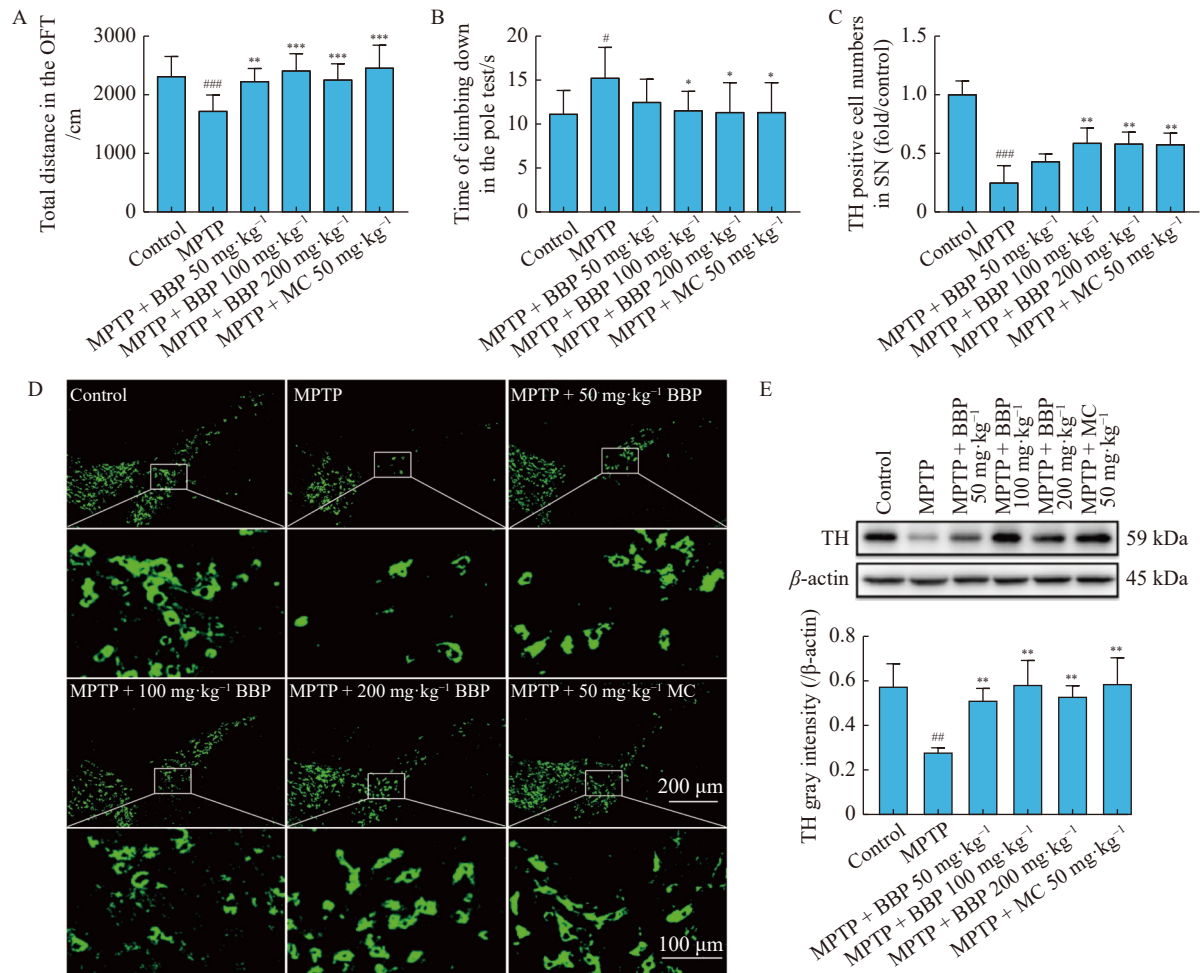


Fig. 1 BBP alleviates motor dysfunction and dopaminergic neurodegeneration in MPTP-induced PD mice. (A) Total distance travelled by mice in the open field test ($n = 12$). (B) Time of climbing down spent by mice in the pole test ($n = 12$). (C–D) Representative immunofluorescence staining for TH and TH-positive cell counts in the SN ($n = 4$). (E) Protein expression of TH in the SN ($n = 4$). Scale bar: 200 μm . Data are expressed as the mean \pm SEM. [#] $P < 0.05$, ^{##} $P < 0.01$, ^{###} $P < 0.001$ vs the control group; ^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$ vs the MPTP group.

pole test ($P < 0.05$). MPTP induction significantly reduced the total travel distance of mice in the open field test ($P < 0.001$). In addition, MPTP induction significantly reduced the levels of TH protein and the number of TH positive cells in mouse SN ($P < 0.01$, $P < 0.001$). Compared with MPTP, BBP (100 and 200 mg·kg⁻¹) and MC (50 mg·kg⁻¹) treatments remarkably shortened the time of climbing down by the mice ($P < 0.05$). BBP (100, 200 mg·kg⁻¹) and MC treatments greatly increased the total travel distance of the mice, as well as the levels of TH and the number of TH positive cells in the SN ($P < 0.01$ or $P < 0.001$). Collectively, these findings suggest that BBP treatment ameliorates MPTP-induced motor dysfunction and dopaminergic neurodegeneration in mice.

tion in mice.

BBP suppresses astrocytic inflammation and modulates the TGR5/AKT/NF- κ B signaling pathway in the SN of MPTP-induced mice

Astrocytes are the key regulators of neuroinflammation, and up-regulation of astrocyte marker GFAP expression is a typical indicator of CNS diseases [39]. As shown in Figs. 2A–2C, 3A–3B and Supplementary Fig. 1, the number of GFAP positive cells and the levels of GFAP, IBA1, iNOS and COX2 proteins significantly increased in the SN of MPTP-induced mice ($P < 0.01$ or $P < 0.001$), compared with the control group. The levels of TGR5 protein were significantly reduced ($P < 0.01$). Meanwhile, the phosphorylation of

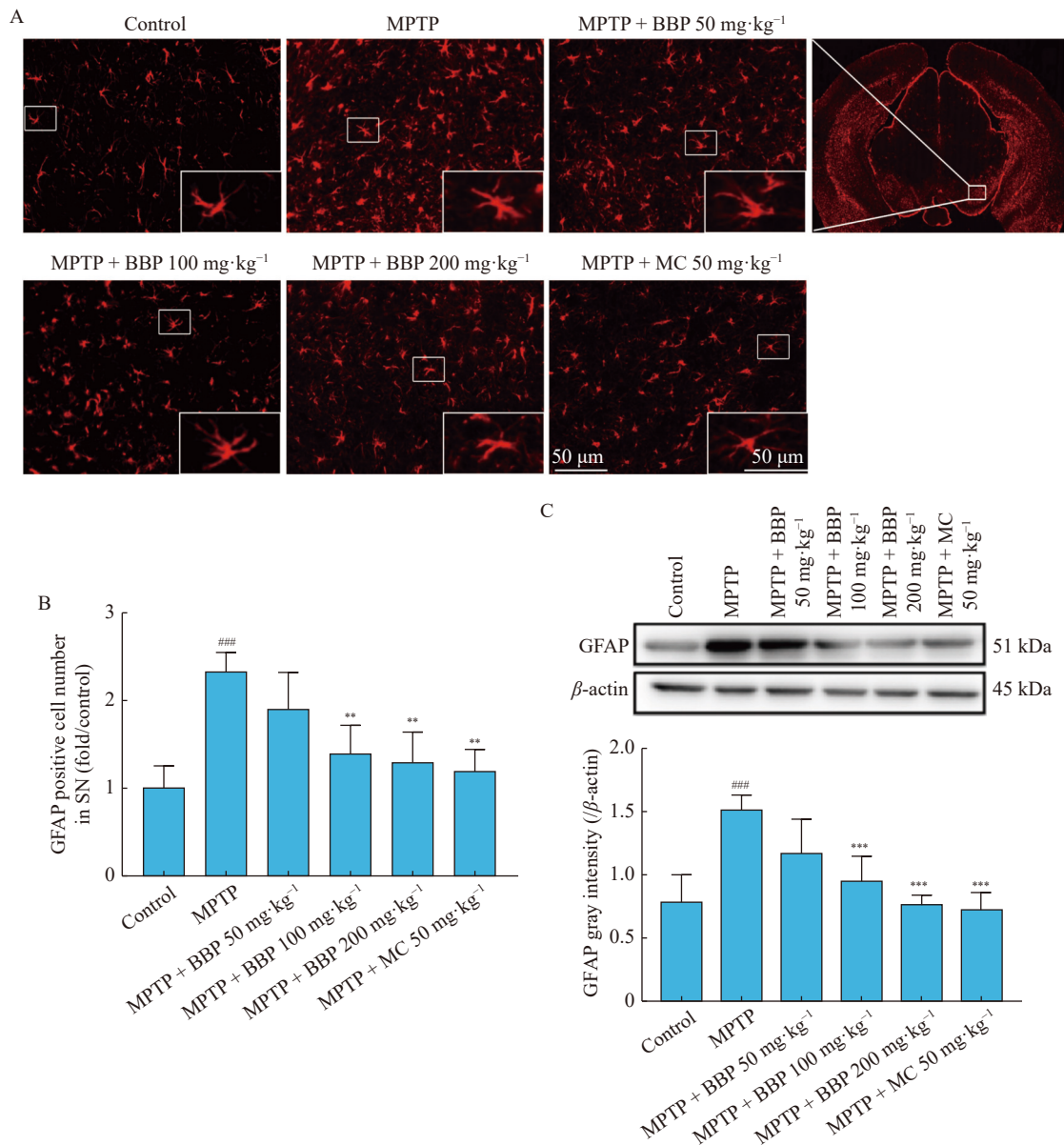


Fig. 2 BBP reduces the number of GFAP positive cells in the SN of MPTP-induced mice. (A–B) Representative immunofluorescence staining for GFAP and GFAP-positive cell counts in the SN. (C) Protein expression of GFAP in the SN. Scale bar: 50 μ m. Data are expressed as the mean \pm SEM ($n = 4$). ^{###} $P < 0.001$ vs the control group; ^{**} $P < 0.01$, ^{***} $P < 0.001$ vs the MPTP group.

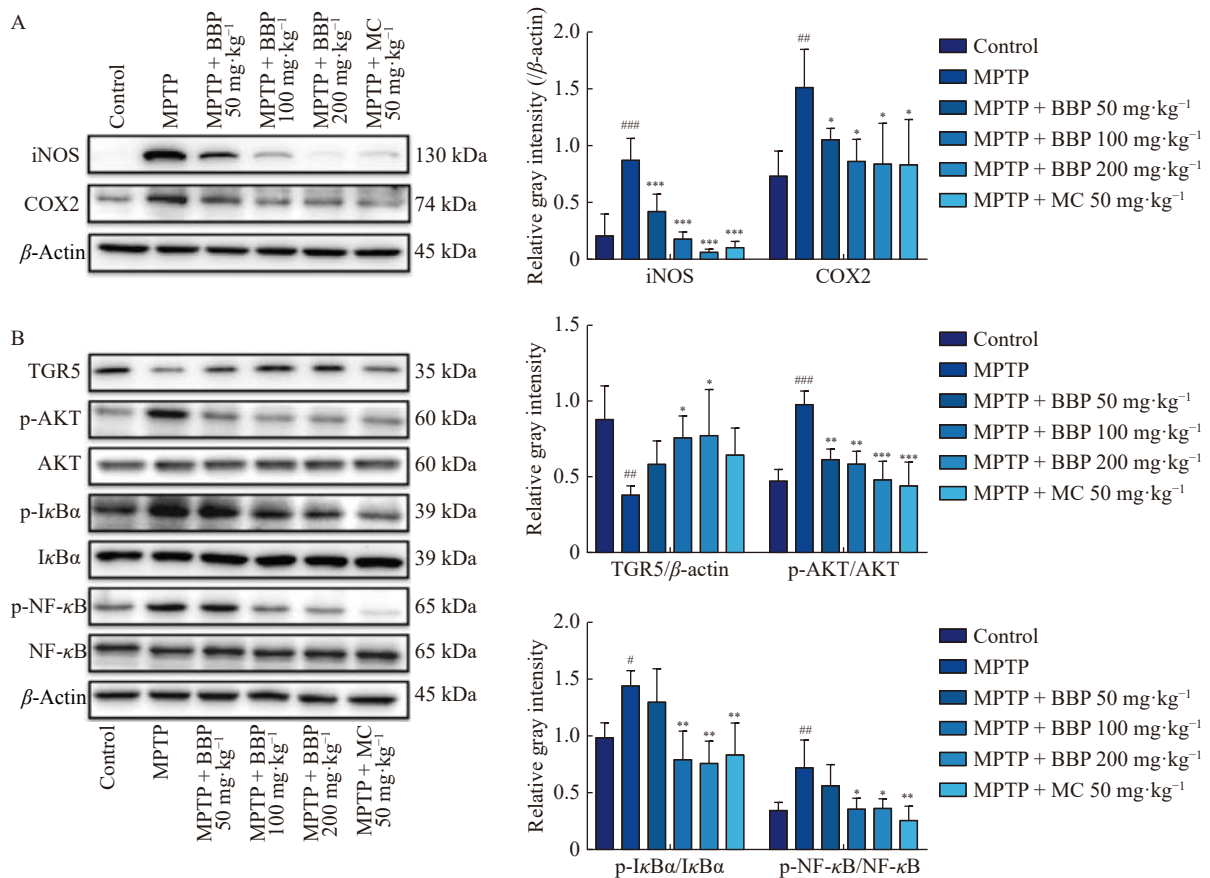


Fig. 3 BBP suppresses inflammation in the SN of MPTP-induced mice via the TGR5-mediated AKT/NF- κ B signaling pathway. (A) Protein expression of iNOS and COX2 in the SN. (B) Protein expression of TGR5, phosphorylated AKT, I κ B α and NF- κ B. Data are expressed as the mean \pm SEM ($n = 4$). [#] $P < 0.05$, ^{##} $P < 0.01$, ^{###} $P < 0.001$ vs the control group; ^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$ vs the MPTP group.

AKT, I κ B α and NF- κ B were markedly up-regulated ($P < 0.05$, $P < 0.01$ or $P < 0.001$). In contrast, both BBP (100 and 200 mg·kg⁻¹) and MC (50 mg·kg⁻¹) treatments considerably decreased the number of GFAP positive cells and the levels of GFAP, IBA1, iNOS and COX2 proteins ($P < 0.05$, $P < 0.01$ or $P < 0.001$). BBP (100 and 200 mg·kg⁻¹) treatment markedly prevented the reduction of TGR5 protein induced by MPTP in mice ($P < 0.05$). In addition, both BBP and MC treatments greatly lessened the phosphorylation of AKT, I κ B α and NF- κ B ($P < 0.05$, $P < 0.01$ or $P < 0.001$).

BBP attenuates inflammation in LPS-induced C6 cells

As shown in Figs. 4A–4B, the cell viability of C6 cells treated with BBP for 24 h did not significantly change. Meanwhile, both BBP (25, 50, and 100 μ g·mL⁻¹) and DEX (1 μ mol·L⁻¹) did not affect the cell viability of C6 cells stimulated by LPS. Compared with the control group, the release of NO in LPS-stimulated C6 cells was markedly elevated (Fig. 4C, $P < 0.001$). Furthermore, the protein levels of GFAP, iNOS and COX2 and the mRNA levels of GFAP, iNOS, COX2, IL-1 β , IL-6 and TNF- α remarkably increased (Figs. 4D–4F, $P < 0.001$). In contrast, pretreatment with BBP (25, 50, 100 μ g·mL⁻¹) and DEX (1 μ mol·L⁻¹) greatly reduced the release of NO, the protein levels of GFAP, iNOS and COX2

and the mRNA levels of GFAP, iNOS, COX2, IL-1 β , IL-6 and TNF- α ($P < 0.05$, $P < 0.01$ or $P < 0.001$).

BBP inhibits inflammation responses via the TGR5/AKT/NF κ B signaling pathway in LPS-induced C6 cells

The pCRE-luc plasmid contains a cAMP response element (CRE). TGR5 is the only known G-protein receptor that responds to bile acid by producing cAMP [40]. To determine the effect of BBP on GR5 transcriptional activation, the dual luciferase reporter assay was used. Compared with the control group, both BBP (25, 50, 100 μ g·mL⁻¹) and INT-777 (0.5 μ mol·L⁻¹) treatments significantly stimulated the activation of TGR5 (Fig. 5A, $P < 0.01$ or $P < 0.001$). As shown in Fig. 5B, LPS stimulation greatly promoted the nuclear translocation of NF- κ B in C6 cells, while BBP and DEX pretreatments noticeably prevented the nuclear translocation of NF- κ B. Moreover, LPS treatment greatly enhanced the phosphorylation of AKT, I κ B α and NF- κ B proteins in C6 cells (Fig. 5D, $P < 0.01$ or $P < 0.001$). In contrast, pretreatment with BBP and DEX prevented the increased phosphorylation of AKT, I κ B α and NF- κ B proteins ($P < 0.05$ or $P < 0.01$). However, the changes of TGR5 protein level in C6 cells treated by LPS were not obvious, nor was the change of TGR5 protein level in LPS-treated C6 cells when BBP was added (Fig. 5C).

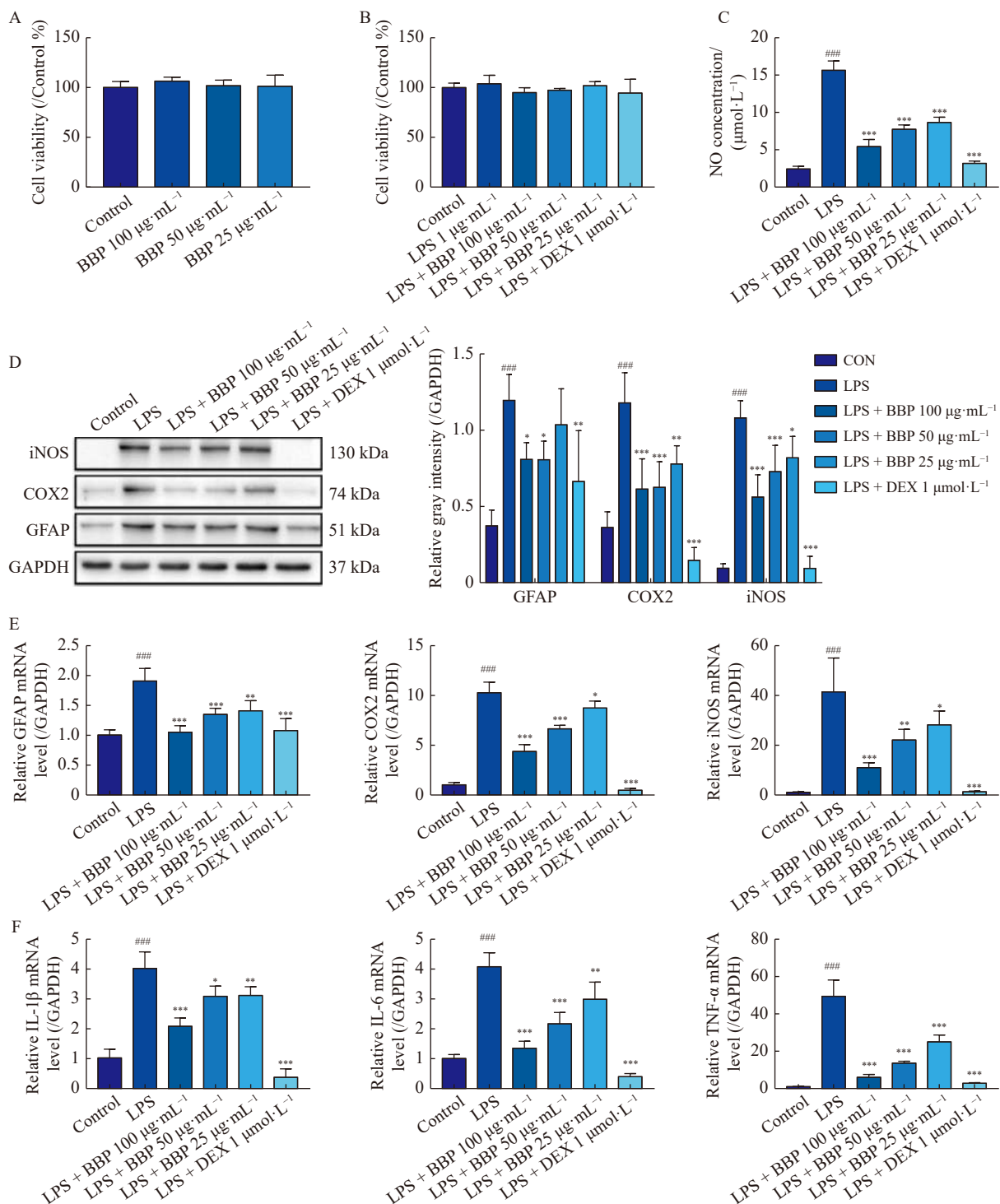


Fig. 4 BBP decreases LPS-induced inflammation in C6 cells. (A) Cell viability of C6 cells treated with BBP at different doses (25, 50, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$) for 24 h. (B) Cell viability of C6 cells treated with LPS (1 $\mu\text{g}\cdot\text{mL}^{-1}$) and BBP at different doses (25, 50, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$) for 24 h. (C) NO production by the cells. (D) Protein expression of GFAP, COX2 and iNOS. (E) mRNA expression of GFAP, COX2 and iNOS. (F) mRNA expression of IL-1 β , IL-6 and TNF- α . Data are expressed as the mean \pm SEM ($n = 4$).
$P < 0.001$ vs the control group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs the LPS group.

To further confirm the anti-inflammatory mechanism of BBP, triamterene, an inhibitor of TGR5, was used to test whether BBP attenuated LPS-induced astrocytic inflammation *via* TGR5. As shown in Figs. 6A–6C, addition of tri-

amterene to C6 cells induced by LPS markedly increased the release of NO, the levels of iNOS and COX2 proteins and the phosphorylation of AKT, I κ B α and NF- κ B ($P < 0.05$, $P < 0.01$ or $P < 0.001$). Compared with the LPS + BBP group, tri-

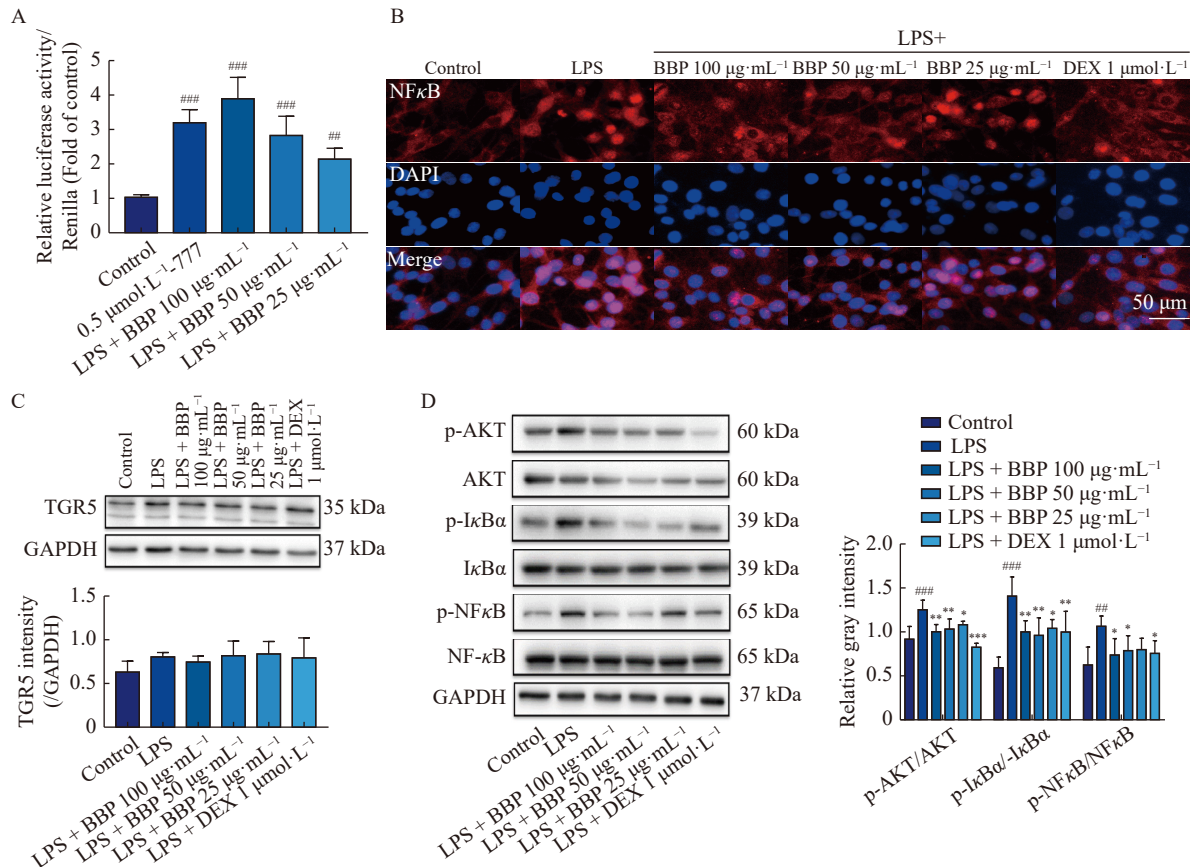


Fig. 5 BBP inhibits the TGR5/AKT/NF- κ B signaling pathway in LPS-treated C6 cells. (A) Activation of TGR5 stimulated by BBP. Untreated HEK 293T cells were used as the negative control, and INT-777 ($0.5 \mu\text{mol}\cdot\text{L}^{-1}$) was used as the positive control. (B) Representative images of NF- κ B (red) staining in C6 cells induced by LPS. The nucleus were stained with 4,6-diaminido-2-phenylindole (DAPI, blue). (C) Protein expression of TGR5. (D) Protein expression of phosphorylated AKT, I κ B α and NF- κ B ($n = 4$). Scale bar: 50 μm . Data are expressed as the mean \pm SEM ($n = 4$). ^{##} $P < 0.01$, ^{###} $P < 0.001$ vs the control group; ^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$ vs the LPS group.

amterene strikingly attenuated the effect of BBP treatment on reducing the release of NO ($P < 0.01$). Meanwhile, the effect of BBP treatment on reducing the protein levels of iNOS and COX2 and the phosphorylation of AKT, I κ B α and NF- κ B proteins were also significantly weakened by triamterene ($P < 0.05$). These findings implicate that BBP inhibits astrocytic neuroinflammation *via* the TGR5-mediated signaling pathway.

Discussion

Neuroinflammation is a major feature of various neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Alzheimer's disease (AD), Huntington's disease (HD) and Parkinson's disease (PD) [41]. More and more studies have shown that inhibiting neuroinflammation can alleviate the symptoms of PD. For example, curcumin ameliorated motor deficits and neuroinflammation in PD mice [42]. Shikonin improved the apoptosis of dopaminergic neurons in the animal models of PD by inhibiting neuroinflammation [43]. Catechin and quercetin reduced nerve damage in experimental PD rats by regulating inflammation [44]. Neuroinflammation caused by activation of astro-

cytes has been revealed to promote the development of PD [45]. In the present study, BBP ameliorated dyskinesia, suppressed astrocyte activation, and inhibited the expression of GFAP, iNOS and COX2 proteins in PD mice. In addition, BBP showed similar anti-neuroinflammatory effect on LPS-induced C6 cells. These findings indicate that BBP plays an anti-neuroinflammatory role *in vitro* and *in vivo*, and provides evidence for the use of BBP in the treatment of PD.

As a nuclear transcription factor, NF- κ B can initiate and regulate a variety of inflammatory processes in glial cells and plays an indispensable role in neurodegenerative diseases [46]. Activation of the NF- κ B signaling pathway can induce the activation of astrocytes and up-regulate the expression of inflammatory cytokines [47]. Overexpression of aquaporin 2 activated rat astrocytes *via* the NF κ B signaling pathway and released a large number of pro-inflammatory cytokines, which then aggravated inflammatory response after intracerebral hemorrhage [48]. Curcumin and NF- κ B p65 siRNA suppressed astrocytes activation by inhibiting the NF- κ B signaling pathway and alleviated spinal cord injury in rats [49]. AKT promoted the phosphorylation of I κ B α through activation of the I κ B kinase complex (IKK), leading to the dissociation of

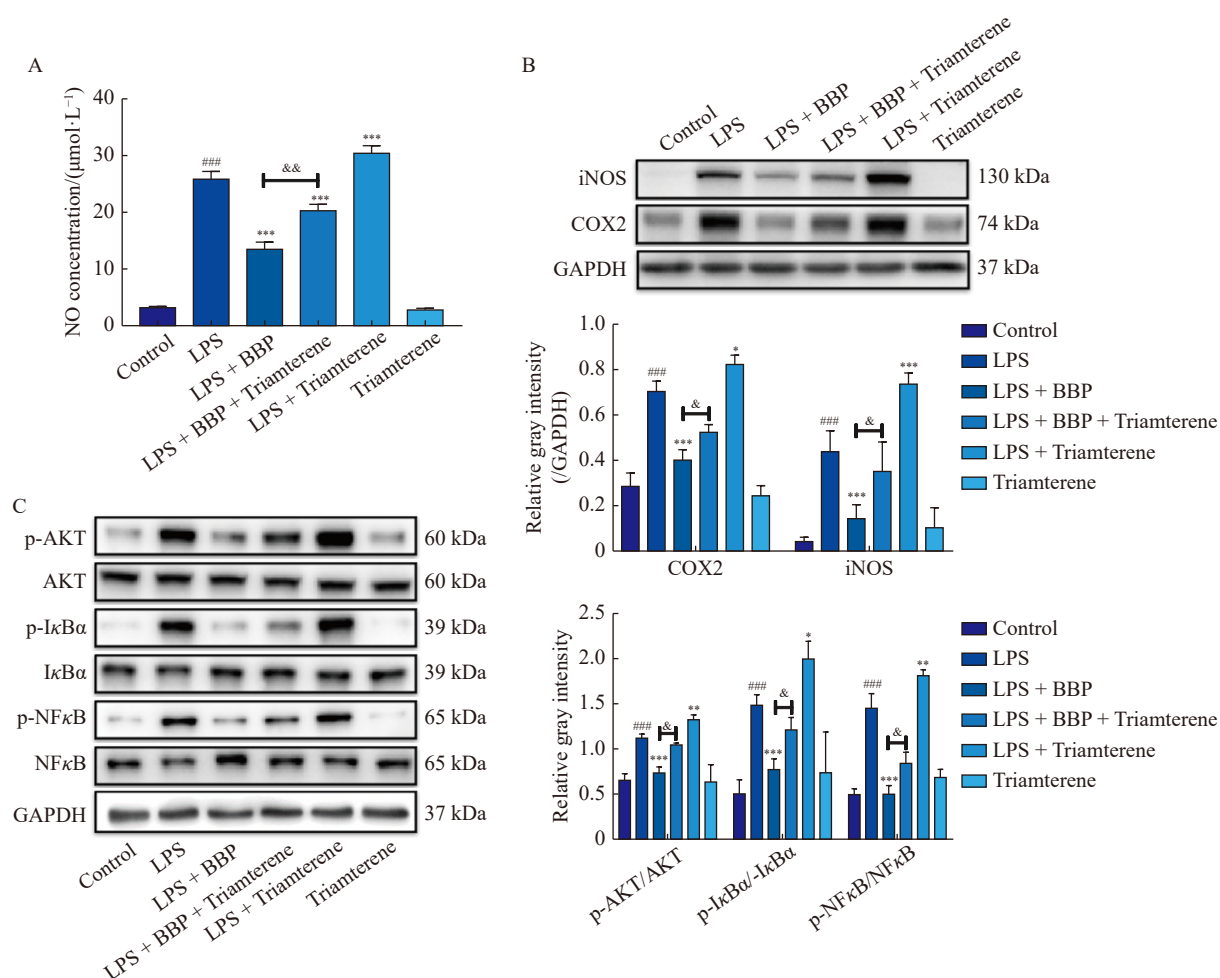


Fig. 6 Anti-inflammatory effect of BBP is blocked by TGR5 inhibitor in C6 cells induced by LPS. (A) NO production by the cells. (B) Protein expression of COX2 and iNOS. (C) Protein expression of phosphorylated AKT, I κ B α and NF- κ B. LPS, 1 $\mu\text{g}\cdot\text{mL}^{-1}$; BBP, 100 $\mu\text{g}\cdot\text{mL}^{-1}$. Data are expressed as the mean \pm SEM ($n = 4$). ^{###} $P < 0.001$ vs the control group; ^{*} $P < 0.05$, ^{**} $P < 0.001$, ^{***} $P < 0.001$ vs the LPS group; [&] $P < 0.05$, ^{&&} $P < 0.01$ vs the LPS + BBP group.

NF κ B from the I κ B α /NF- κ B complex. Once released and phosphorylated, NF- κ B entered the nucleus and activated the inflammatory process [50]. In this study, BBP effectively inhibited the nuclear translocation of NF- κ B and attenuated the phosphorylation of AKT, I κ B α and NF- κ B proteins in LPS-stimulated C6 cells. Similarly, it decreased the phosphorylation of AKT, I κ B α and NF κ B proteins in the SN of MPTP-induced mice. These findings suggest that BBP suppresses astrocytic inflammation *via* the AKT/I κ B α /NF- κ B signaling pathway.

TGR5 is a major membrane receptor of bile acids, which can play anti-inflammatory, anti-apoptotic and neuroprotective effect after activation by certain bile acids [51]. TGR5 activation can reduce BBB breakdown after ischemic stroke [52], and attenuate microglial activation and acute injury in hepatic encephalopathy [53]. TGR5 agonist alleviated atherosclerosis by inhibiting the nuclear migration of NF- κ B and the phosphorylation of I κ B α in macrophages [54]. Recent studies by our group have also shown that hyodeoxycholic acid inhibits LPS-induced inflammation of the CNS by regulating

the TGR5/AKT/NF- κ B signaling pathway [55]. In this study, BBP elevated the levels of TGR5 protein and inhibited the AKT/NF- κ B signaling pathway in MPTP-induced mice. *In vitro*, the expression of TGR5 protein did not significantly change, but BBP significantly activated TGR5 and suppressed the AKT/NF- κ B signaling pathway. After administration of TGR5 inhibitor, the anti-neuroinflammatory effect of BBP on astrocytes was antagonized. These findings verify that BBP may play an anti-neuroinflammatory role by suppressing the AKT/NF- κ B signaling pathway *via* TGR5 (Fig. 7).

One of the limitations of the present study is lack of studies concerning TGR5 antagonist, such as triamterene, in PD mice. Indeed, triamterene is mainly used to treat various types of edema, such as ascites caused by heart failure, cirrhosis and chronic nephritis, and water and sodium retention during glucocorticoid treatment. Due to the potentially unspecific reactions *in vivo*, triamterene treatment in PD mice is equivalent to giving a new drug, which may not fully explain the role of TGR5 in the therapeutic effect of BBP on PD

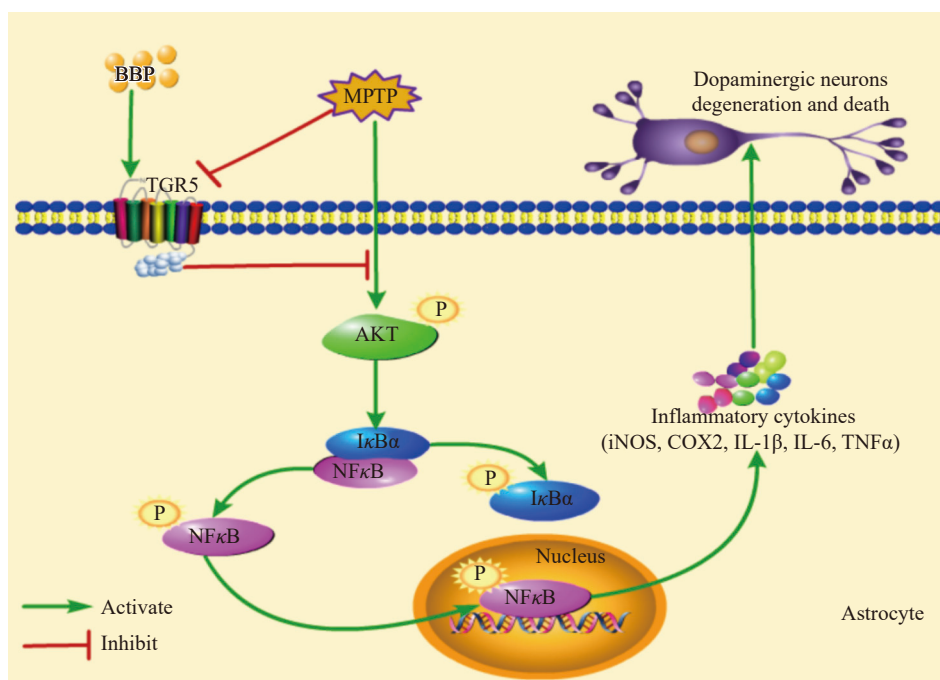


Fig. 7 BBP alleviates the progression of PD mice by suppressing astrocyte activation via the TGR5/AKT/NF-κB signaling pathway

mice. Instead, it is necessary to use TGR5 knockout method, especially astrocytic TGR5 knockout mice, in order to fully elucidate the role of TGR5 in the beneficial effect of BBP *in vivo* in the future.

In sum, BBP alleviates the progression of PD mice, which may be closely related to the suppression of astrocytic neuroinflammation by regulating the TGR5-mediated AKT/NF-κB signaling pathway.

Abbreviations

GFAP: Glial fibrillary acidic protein; IBA1: Adapter molecules 1; COX2: Cyclooxygenase 2; iNOS: Inducible nitric oxide synthase; NF-κB: Nuclear factor κB; IκBα: Inhibitor of NF-κB; AKT: Protein kinase B; LPS: Lipopolysaccharide

References

- [1] Qiu J, Chen Y, Zhuo J, et al. Urolithin A promotes mitophagy and suppresses NLRP3 inflammasome activation in lipopolysaccharide-induced BV2 microglial cells and MPTP-induced Parkinson's disease model [J]. *Neuropharmacology*, 2022, **207**: 108963.
- [2] Li H, Feng Y, Chen Z, et al. Pepper component 7-ethoxy-4-methylcoumarin, a novel dopamine D2 receptor agonist, ameliorates experimental Parkinson's disease in mice and *Caenorhabditis elegans* [J]. *Pharmacol Res*, 2021, **163**: 105220.
- [3] Zhu J, Gao W, Shan X, et al. Apelin-36 mediates neuroprotective effects by regulating oxidative stress, autophagy and apoptosis in MPTP-induced Parkinson's disease model mice [J]. *Brain Res*, 2020, **1726**: 146493.
- [4] Poewe W, Seppi K, Tanner CM, et al. Parkinson disease [J]. *Nat Rev Dis Primers*, 2017, **3**: 17013.
- [5] Sun SY, An CN, Pu XP. DJ-1 protein protects dopaminergic neurons against 6-OHDA/MG-132-induced neurotoxicity in rats [J]. *Brain Res Bull*, 2012, **88**(6): 609-616.
- [6] Al-Nusaif M, Lin Y, Li T, et al. Advances in NURR1-regulated neuroinflammation associated with Parkinson's disease

- [J]. *Int J Mol Sci*, 2022, **23**(24): 16184.
- [7] Hirsch EC, Vyas S, Hunot S. Neuroinflammation in Parkinson's disease [J]. *Parkinsonism Relat Disord*, 2012, **18**(Suppl 1): S210-S212.
- [8] Wang ZL, Yuan L, Li W, et al. Ferroptosis in Parkinson's disease: glia-neuron crosstalk [J]. *Trends Mol Med*, 2022, **28**(4): 258-269.
- [9] Oduola-Akande MD, Ishola IO, Olubodun-Obadun TG, et al. Glimepiride prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced dopamine neurons degeneration through attenuation of glia activation and oxidative stress in mice [J]. *Neurotox Res*, 2023, **41**: 212-223.
- [10] Sharma M, Malim FM, Goswami A, et al. Neuroprotective effect of swertiamarin in a rotenone model of Parkinson's disease: role of neuroinflammation and alpha-synuclein accumulation [J]. *ACS Pharmacol Transl Sci*, 2023, **6**(1): 40-51.
- [11] Cao Q, Luo S, Yao W, et al. Suppression of abnormal α-synuclein expression by activation of BDNF transcription ameliorates Parkinson's disease-like pathology [J]. *Mol Ther Nucleic Acids*, 2022, **29**: 1-15.
- [12] Saijo K, Winner B, Carson CT, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death [J]. *Cell*, 2009, **137**(1): 47-59.
- [13] Song N, Wang J, Jiang H, et al. Astroglial and microglial contributions to iron metabolism disturbance in Parkinson's disease [J]. *Biochim Biophys Acta Mol Basis Dis*, 2018, **1864**(3): 967-973.
- [14] Tsai SJ, Chao CY, Yin MC. Preventive and therapeutic effects of caffeic acid against inflammatory injury in striatum of MPTP-treated mice [J]. *Eur J Pharmacol*, 2011, **670**(2-3): 441-447.
- [15] Chen Y, Li Y, Li C, et al. Dexmedetomidine alleviates pain in MPTP-treated mice by activating the AMPK/mTOR/NF-κB pathways in astrocytes [J]. *Neurosci Lett*, 2022, **791**: 136933.
- [16] Yun SP, Kam TI, Panicker N, et al. Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease [J]. *Nat Med*, 2018, **24**(7): 931-938.
- [17] Chávez-Talavera O, Tailleux A, Lefebvre P, et al. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease [J].

- Gastroenterology*, 2017, **152**(7): 1679-1694.e1673.
- [18] Sun L, Cai J, Gonzalez FJ. The role of farnesoid X receptor in metabolic diseases, and gastrointestinal and liver cancer [J]. *Nat Rev Gastroenterol Hepatol*, 2021, **18**(5): 335-347.
- [19] Keitel V, Görg B, Bidmon HJ, et al. The bile acid receptor TGR5 (Gpbar-1) acts as a neurosteroid receptor in brain [J]. *Glia*, 2010, **58**(15): 1794-1805.
- [20] Yanguas-Casás N, Barreda-Manso MA, Nieto-Sampedro M, et al. TUDCA: an agonist of the bile acid receptor GPBAR1/TGR5 with anti-inflammatory effects in microglial cells [J]. *J Cell Physiol*, 2017, **232**(8): 2231-2245.
- [21] Guo C, Chen WD, Wang YD. TGR5, not only a metabolic regulator [J]. *Front Physiol*, 2016, **7**: 646.
- [22] Sorrentino G, Perino A, Yildiz E, et al. Bile acids signal via TGR5 to activate intestinal stem cells and epithelial regeneration [J]. *Gastroenterology*, 2020, **159**(3): 956-968.e958.
- [23] Hu J, Wang C, Huang X, et al. Gut microbiota-mediated secondary bile acids regulate dendritic cells to attenuate autoimmune uveitis through TGR5 signaling [J]. *Cell Rep*, 2021, **36**(12): 109726.
- [24] Perino A, Pols TW, Nomura M, et al. TGR5 reduces macrophage migration through mTOR-induced C/EBP β differential translation [J]. *J Clin Invest*, 2014, **124**(12): 5424-5436.
- [25] Lewis ND, Patnaude LA, Pelletier J, et al. A GPBAR1 (TGR5) small molecule agonist shows specific inhibitory effects on myeloid cell activation *in vitro* and reduces experimental autoimmune encephalitis (EAE) *in vivo* [J]. *PLoS One*, 2014, **9**(6): e100883.
- [26] Jiang KY, Zhang Y, Ye XL, et al. Bear bile powder attenuates senecionine-induced hepatic sinusoidal obstruction syndrome in mice [J]. *Chin J Nat Med*, 2022, **20**(4): 270-281.
- [27] Zhao JY, Lin W, Zhuang QC, et al. Bear bile powder inhibits angiogenesis *in vivo* and *in vitro* [J]. *Chin J Integr Med*, 2015, **21**(5): 369-375.
- [28] Zhou CF, Gao GJ, Liu Y. Advances in studies on bear bile powder [J]. *China J Chin Mater Med*, 2015, **40**(7): 1252-1258.
- [29] Lei Z, Xudong W, Wei M. Effects of taurochenodeoxycholic acid on Ca²⁺/CaM signalling mediated by the TGR5 signalling pathway [J]. *Pharmazie*, 2016, **71**(7): 390-393.
- [30] Maczewsky J, Kaiser J, Gresch A, et al. TGR5 activation promotes stimulus-secretion coupling of pancreatic β -cells via a PKA-dependent pathway [J]. *Diabetes*, 2019, **68**(2): 324-336.
- [31] Wu X, Liu C, Chen L, et al. Protective effects of tauroursodeoxycholic acid on lipopolysaccharide-induced cognitive impairment and neurotoxicity in mice [J]. *Int Immunopharmacol*, 2019, **72**: 166-175.
- [32] Zheng M, Li YY, Wang GF, et al. Protective effect of cultured bear bile powder against dimethylnitrosamine-induced hepatic fibrosis in rats [J]. *Biomed Pharmacother*, 2019, **112**: 108701.
- [33] Sun X, Xue H, Zan B, et al. Anti-convulsant effects of cultures bear bile powder in febrile seizure via regulation of neurotransmission and inhibition of neuroinflammation [J]. *J Ethnopharmacol*, 2020, **262**: 112998.
- [34] Zhu H, Wang G, Bai Y, et al. Natural bear bile powder suppresses neuroinflammation in lipopolysaccharide-treated mice via regulating TGR5/AKT/NF- κ B signaling pathway [J]. *J Ethnopharmacol*, 2022, **289**: 115063.
- [35] Kraeuter AK, Guest PC, Saranyai Z. The open field test for measuring locomotor activity and anxiety-like behavior [J]. *Methods Mol Biol*, 2019, **1916**: 99-103.
- [36] Zhang QS, Heng Y, Mou Z, et al. Reassessment of subacute MPTP-treated mice as animal model of Parkinson's disease [J]. *Acta Pharmacol Sin*, 2017, **38**(10): 1317-1328.
- [37] Qi Y, Shi L, Duan G, et al. Taurochenodeoxycholic acid increases cAMP content via specially interacting with bile acid receptor TGR5 [J]. *Molecules*, 2021, **26**(23).
- [38] Zheng S, Zou Y, Xie X, et al. Development and validation of a stromal immune phenotype classifier for predicting immune activity and prognosis in triple-negative breast cancer [J]. *Int J Cancer*, 2020, **147**(2): 542-553.
- [39] Colombo E, Farina C. Astrocytes: key regulators of neuroinflammation [J]. *Trends Immunol*, 2016, **37**(9): 608-620.
- [40] Watanabe M, Houten SM, Matak C, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation [J]. *Nature*, 2006, **439**(7075): 484-489.
- [41] Ransohoff RM. How neuroinflammation contributes to neurodegeneration [J]. *Science*, 2016, **353**(6301): 777-783.
- [42] Cui C, Han Y, Li H, et al. Curcumin-driven reprogramming of the gut microbiota and metabolome ameliorates motor deficits and neuroinflammation in a mouse model of Parkinson's disease [J]. *Front Cell Infect Microbiol*, 2022, **12**: 887407.
- [43] Guo L, Li Y, Li W, et al. Shikonin ameliorates oxidative stress and neuroinflammation via the Akt/ERK/JNK/NF- κ B signalling pathways in a model of Parkinson's disease [J]. *Clin Exp Pharmacol Physiol*, 2022, **49**(11): 1221-1231.
- [44] Josiah SS, Famusiwa CD, Crown OO, et al. Neuroprotective effects of catechin and quercetin in experimental Parkinsonism through modulation of dopamine metabolism and expression of IL-1 β , TNF- α , NF- κ B, I κ B, and p53 genes in male Wistar rats [J]. *Neurotoxicology*, 2022, **90**: 158-171.
- [45] Kam TI, Hinkle JT, Dawson TM, et al. Microglia and astrocyte dysfunction in parkinson's disease [J]. *Neurobiol Dis*, 2020, **144**: 105028.
- [46] Phani S, Loike JD, Przedborski S. Neurodegeneration and inflammation in Parkinson's disease [J]. *Parkinsonism Relat Disord*, 2012, **18**(S1): S207-S209.
- [47] Zhang T, Zheng YD, Jiao M, et al. Nuclear factor- κ B signaling mediates antimony-induced astrocyte activation [J]. *Biomed Environ Sci*, 2021, **34**(1): 29-39.
- [48] Deng S, Chen X, Lei Q, et al. AQP2 promotes astrocyte activation by modulating the TLR4/NF κ B-p65 pathway following intracerebral hemorrhage [J]. *Front Immunol*, 2022, **13**: 847360.
- [49] Yuan J, Liu W, Zhu H, et al. Curcumin inhibits glial scar formation by suppressing astrocyte-induced inflammation and fibrosis *in vitro* and *in vivo* [J]. *Brain Res*, 2017, **1655**: 90-103.
- [50] Torrealba N, Vera R, Fraile B, et al. TGF- β /PI3K/AKT/mTOR/NF- κ B pathway. Clinicopathological features in prostate cancer [J]. *Aging Male*, 2020, **23**(5): 801-811.
- [51] Qi YC, Duan GZ, Mao W, et al. Taurochenodeoxycholic acid mediates cAMP-PKA-CREB signaling pathway [J]. *Chin J Nat Med*, 2020, **18**(12): 898-906.
- [52] McMillin M, Grant S, Frampton G, et al. Fractalkine suppression during hepatic encephalopathy promotes neuroinflammation in mice [J]. *J Neuroinflammation*, 2016, **13**(1): 198.
- [53] McMillin M, Frampton G, Tobin R, et al. TGR5 signaling reduces neuroinflammation during hepatic encephalopathy [J]. *J Neurochem*, 2015, **135**(3): 565-576.
- [54] Pols TW, Nomura M, Harach T, et al. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading [J]. *Cell Metab*, 2011, **14**(6): 747-757.
- [55] Zhu H, Bai Y, Wang G, et al. Hyodeoxycholic acid inhibits lipopolysaccharide-induced microglia inflammatory responses through regulating TGR5/AKT/NF- κ B signaling pathway [J]. *J Psychopharmacol*, 2022, **36**(7): 849-859.

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