Article

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Injection of Collagen Binding Domain-Brain Derived Neurotrophic Factor Promotes SIRT1 Expression: Improving Neuroinflammation in Experimental Subarachnoid Hemorrhage

Xiaosheng Lv[1,*], Yuwen Song[1], Yanyan Shi[1], Yinghong Xue[1], Shuncheng Ge[1], Chao Liu[1]

[1]Department of Neurosurgery, Laoting County Hospital, 063699 Tangshan, Hebei, China
*Correspondence: hbtslxs@126.com (Xiaosheng Lv)
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Background: Subarachnoid hemorrhage (SAH) is a severe cerebrovascular disease, often leading to neuroinflammation and neuronal damage. Activation of the Nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome is closely associated with post-SAH neuroinflammation, while activation of Nicotinamide Adenine Dinucleotide (NAD)-dependent deacetylase sirtuin-1 (SIRT1) has neuroprotective effects. This study aimed to investigate the impact of injectable Collagen Binding Domain-Brain Derived Neurotrophic Factor (CBD-BDNF) on neuroinflammation and neuronal damage following SAH.

Methods: After establishing the SAH model, experimental animals were divided into three groups: sham surgery group (Sham), SAH group, and SAH+neuroregenerative scaffold (CBD-BDNF treatment) group. Behavioral performance was evaluated using neurofunctional deficit, beam balance, and Y-maze tests. Expression of inflammatory factors and essential proteins was quantitatively analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) kits and immunoblotting. Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) staining was used to assess cell apoptosis. To further investigate the mechanism of action of CBD-BDNF on SIRT1, the model animals were treated with EX527 (SIRT1 inhibitor) for comparative studies.

Results: Neurological deficit tests, CBD-BDNF improves functional outcomes after SAH. Compared to the SAH group, the SAH+neuroregenerative scaffold group showed significantly increased expression of SIRT1 protein and significantly decreased expression of NLRP3, Apoptosis-associated speck-like protein containing a CARD (ASC), and c-caspase-1. The inflammatory cytokines Interleukin-1 beta (IL-1β), IL-6, and IL-18 levels also significantly decreased in the SAH+neuroregenerative scaffold group. Additionally, animals in the SAH+neuroregenerative scaffold group showed better neurofunctional recovery in neurofunctional deficit and beam balance tests. The number of apoptotic cells significantly decreased in the SAH+neuroregenerative scaffold group compared to the SAH group. However, when SIRT1 was inhibited with EX527, the aforementioned neuroprotective effects were reversed, indicating the involvement of CBD-BDNF through SIRT1 activation.

Conclusion: This study demonstrates that injectable CBD-BDNF can significantly alleviate neuroinflammation and neuronal damage resulting from SAH by blocking NLRP3 inflammasome activation and promoting SIRT1 expression. These findings provide a new therapeutic strategy for neuroprotection after SAH and reveal the mechanism of action of CBD-BDNF as a potential therapeutic agent. Future research will further explore the long-term efficacy and safety of CBD-BDNF.

Keywords: SAH; CBD-BDNF; NLRP3 inflammasome; SIRT1; neuroinflammation

Introduction

Subarachnoid hemorrhage (SAH) is a common and severe cerebrovascular disease known for its high mortality and disability rates [1–3]. Complications following SAH, particularly early brain injury (EBI) and cerebral vasospasm (CVS), are the major contributors to poor patient outcomes [4,5]. Recent studies have indicated that activation of the Nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome plays a critical role in the post-SAH inflammatory response, while activation of sirtuin-1 (SIRT1) exerts neuroprotective effects [6–8]. However, current pharmacological treatments for SAH are limited to symptomatic relief, lacking effective targeted therapies for inflammation and neuroprotection [9].

In recent years, researchers have extensively investigated the neuroinflammatory mechanisms following SAH [10]. A series of studies have demonstrated that activation of the NLRP3 inflammasome is a key factor in inducing neuroinflammation, participating in the release of inflammatory cytokines, including Interleukin-1 beta (IL-1β) and IL-18 [11,12]. Simultaneously, activation of SIRT1 has been demonstrated to exert neuroprotective effects by

deacetylating Nuclear Factor Kappa κB (NF-κB) and inhibiting the expression of inflammatory cytokines [13–15]. Furthermore, Brain Derived Neurotrophic Factor (BDNF) has been widely studied for its crucial role in neurorepair and regeneration [16,17]. However, the clinical application of BDNF was limited by its instability *in vivo* and ability to penetrate the blood-brain barrier. Recently, a novel Collagen Binding Domain-Brain Derived Neurotrophic Factor (CBD-BDNF) has been developed, enhancing its stability and local concentration in the brain through the collagen-binding domain [18–21].

Despite demonstrating potential in other neurological disease models, the effects and mechanisms of CBD-BDNF in the SAH model remain unclear. Specifically, further research is needed to determine whether CBD-BDNF can improve EBI and CVS following SAH by modulating the activity of NLRP3 inflammasome and SIRT1. Additionally, there is a lack of in-depth research on the specific mechanisms of CBD-BDNF in neuronal damage following SAH and its molecular interactions with NLRP3 inflammasome and SIRT1.

Given the aforementioned background, this study aims to investigate the neuroprotective effects of injectable CBD-BDNF in an experimental SAH model, particularly its inhibitory effect on NLRP3 inflammasome and its promotive effect on SIRT1. Through *in vivo* experiments, we will assess the impact of CBD-BDNF on EBI and CVS following SAH and explore its mechanisms of action using molecular biology techniques. The significance of this study lies in the potential for CBD-BDNF to effectively ameliorate post-SAH neuroinflammation and neuronal damage, potentially serving as a novel therapeutic strategy for SAH and improving patient outcomes while reducing disability rates.

Materials and Methods

SAH Animal Model

SPF-grade healthy male C57BL/6 mice, weighing 20–22 g, were provided by Beijing Huafukang Biotechnology Co., Ltd. (Beijing, China), with an animal license number: SCXK (Jing) 2019-0008. 12 mice were divided into four groups: the Sham group, SAH group, SAH+CBD-BDNF group, and SAH+CBD-BDNF+EX527 group, in a random manner. Mice in the SAH group were administered pentobarbital (40 mg/kg) through an intraperitoneal injection for anesthesia. Subsequently, the left common carotid artery and its branches were uncovered. The distal section of the external carotid artery was cut, and a 4-0 single-strand nylon thread was introduced and moved forward into the internal carotid artery until encountering resistance at the point where the anterior cerebral artery and the middle cerebral artery meet. The suture was further advanced by approximately 3–5 mm to induce arterial perforation and SAH, with a 10-second delay. The Sham group underwent an identical procedure without any perforation. The SAH+CBD-BDNF group refers to the group where our laboratory synthesized and purified CBD-BDNF (3 μM) is injected into the mouse cerebral cortex for treatment based on the SAH animal model. The SAH+CBD-BDNF+EX527 group refers to the group where SIRT1 inhibitor (EX527) is injected into the mouse cerebral cortex for treatment on the basis of the SAH+CBD-BDNF treatment group. Vital signs such as arterial blood pressure, body temperature, and partial pressure of oxygen (PaO_2) were continuously monitored during the procedure. After the experiment, euthanasia was performed by inhalation of carbon dioxide, with a lethal dose of 50% carbon dioxide concentration. The experimental procedures used in the mice were approved by the Ethics Committee of Laoting County Hospital (Approval No.: LT2018-(C57)-01).

Synthesis of CBD-BDNF

Firstly, a gene construct containing both CBD and BDNF sequences was designed and inserted into an overexpression plasmid (pCMV). Subsequently, the overexpression plasmid carrying the *CBD-BDNF* gene was transfected into human embryonic kidney cells HEK-293 (iCell-h243, Cellverse Bioscience Technology Co., Ltd., Shanghai, China) using a lipid-based transfection reagent. The cells were then cultured for 72 hours at 37°C and 5% CO_2. Subsequently, CBD-BDNF was extracted and purified from the HEK-293 cells using cell lysis and purification techniques. Finally, the purified product was validated. The cell lines involved in this section have completed STR identification, and the mycoplasma detection results were negative.

Neurological Function Deficit Testing

At 24 hours after SAH, the scoring system developed by Fritz *et al.* [22] was used to assess neurological impairments. The scoring system consisted of 6 tests: voluntary movement, limb movement symmetry, forelimb extension, climbing ability, proprioception, and response to touch. Each test was scored from 0 to 3 or 1 to 3, with a total score range of 3 to 18. Lower scores indicated more severe neurological deficits.

Beam Balance Testing

Beam balance testing was employed to assess the animals' balance and coordination. The experimental procedure involved training the animals to traverse a beam and recording their performance on the beam, including duration of stay, gait, etc., followed by quantitative scoring.

Enzyme-Linked Immunosorbent Assay (ELISA) Quantitative Analysis

The levels of IL-1β (EK201B2), IL-6 (EK206HS), and IL-18 (EK218) were analyzed using ELISA. The ELISA kits were all purchased from MULTISCIENCES (LIANKE) Biotech, Co., Ltd. (Hangzhou, China). After diluting the stock solutions of standards, 50 μL of standard

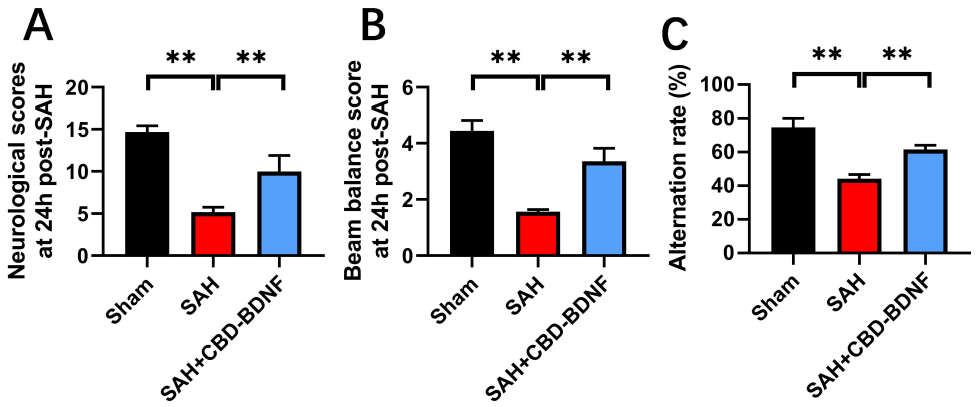

Fig. 1. CBD-BDNF improves short-term and long-term functional outcomes after SAH. (A) Neurological deficit testing at 24 hours post-operation day (POD). (B) Balance beam testing at 24 hours POD. (C) CBD-BDNF improves spatial working memory after SAH through spontaneous alternation tasks in the Y-maze. $^{**}p < 0.01$, n = 3. CBD-BDNF, Collagen Binding Domain-Brain Derived Neurotrophic Factor; SAH, subarachnoid hemorrhage.

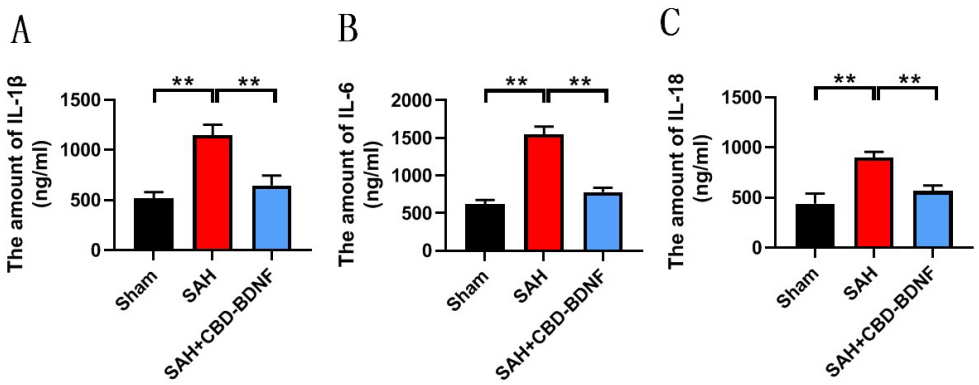

Fig. 2. CBD-BDNF reduces inflammation. (A–C) Quantitative analysis of IL-1β (A), IL-6 (B), and IL-18 (C) levels using ELISA assay kits. $^{**}p < 0.01$, n = 3. IL-1β, Interleukin-1 beta; ELISA, Enzyme-Linked Immunosorbent Assay.

solution and 50 µL of HRP-conjugated streptavidin were added to the standard wells. To conduct the test, 40 µL of the sample was placed into the designated wells, then 10 µL of biotinylated antibody and 50 µL of HRP-conjugated streptavidin were added. Afterward, the dish was placed in an incubator at 37 °C for 60 minutes. Each well was washed with 350 µL of wash buffer. Following the development of color, 50 µL of stop solution was introduced to halt the reaction. Data analysis involved measuring the absorbance at a wavelength of 450 nm for each well using an ELISA reader.

Western Blotting

The tissue samples were mixed and spun (at 1000 revolutions per minute, for 10 minutes, at a temperature of 4 degrees Celsius). The supernatant was further centrifuged, and the protein concentration was determined using a DC protein assay kit with bovine serum albumin standard. The protein was suspended in sample buffer in equal quantities (50 µg), then denatured at 95 °C for 5 minutes before being loaded onto a Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) gel made of sodium dodecyl sulfate-polyacrylamide. Following electrophoresis and transfer to a polyvinylidene difluoride membrane, the membrane underwent blocking with a non-fat milk buffer for 2 hours at a temperature of 4 °C. Subsequently, it was incubated with primary antibodies targeting NLRP3 (ab263899, Abcam, 1:800, Cambridge, United Kingdom), Apoptosis-associated speck-like protein containing a CARD (ASC) (ab283684, Abcam, 1:1000, Cambridge, United Kingdom), and caspase-1 (ab207802, Abcam, 1:1000, Cambridge, United Kingdom), SIRT1 (ab110304, Abcam, 1:1000, Cambridge, United Kingdom), Bax (ab32503, Abcam, 1:1000, Cambridge, United Kingdom), Bcl-2 (ab182858, Abcam, 1:1000, Cambridge, United Kingdom), Cleaved Caspase-1 (4199S, Cell Signaling, 1:1000, Shanghai, China) and GAPDH (ab9485, Abcam, 1:1000, Cambridge, United Kingdom). Afterward, the membrane was incubated with secondary antibodies (Goat anti-rabbit IgG, 1:4000, ab6721, Abcam, Cambridge, United Kingdom) conjugated with horseradish peroxidase at ambient temperature for 1 hour. The densities of pro-

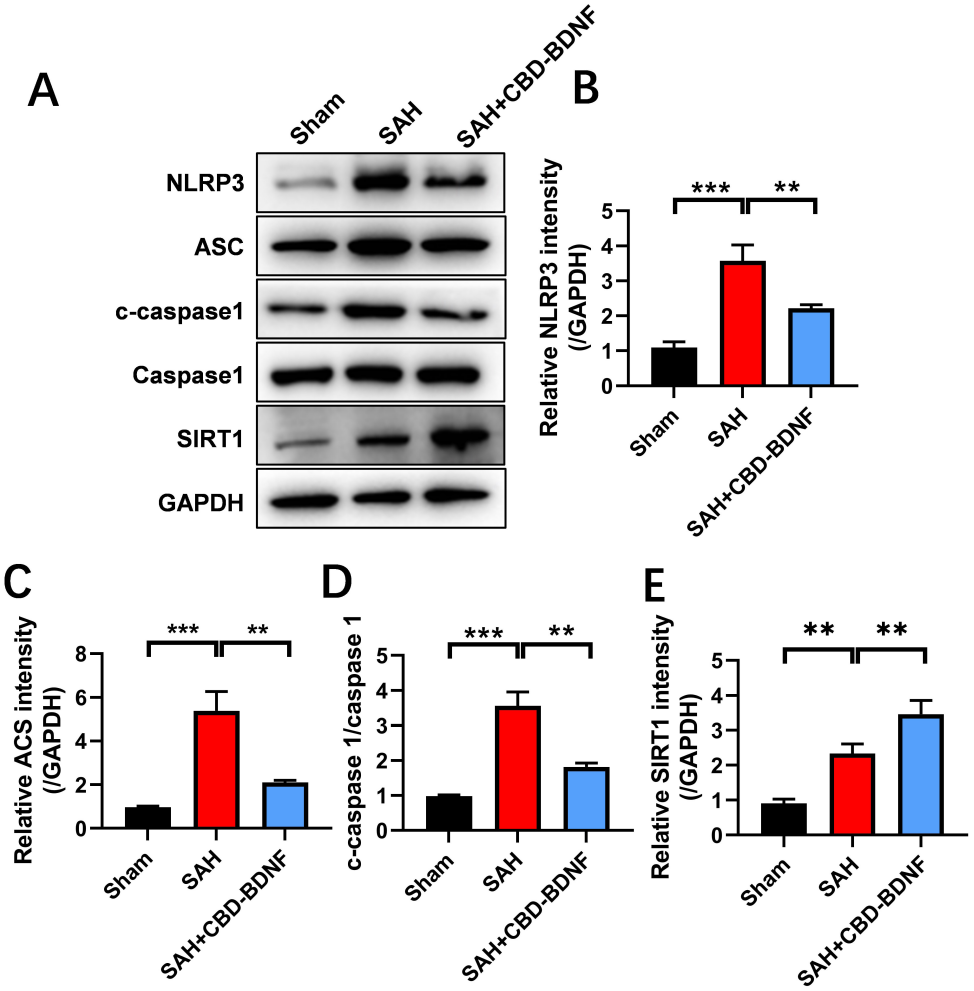

Fig. 3. CBD-BDNF inhibits NLRP3 inflammasome activation and promotes SIRT1 expression. (A) Protein expression of NLRP3, ASC, caspase-1, c-caspase-1, and SIRT1 in the ipsilateral cortex as detected by immunoblotting. (B–E) Quantitative analysis of NLRP3 (B), ASC (C), c-caspase-1 (D), and SIRT1 (E) levels. $^{**}p < 0.01$, $^{***}p < 0.001$, n = 3. ASC, Apoptosis-associated speck-like protein containing a CARD; NLRP3, Nucleotide-binding oligomerization domain (NOD)-like receptor protein 3; SIRT1, sirtuin-1.

tein bands were identified using an imaging system (Tanon-4600, Beijing Junyi Oriental Electrophoresis Equipment Co., Ltd., Beijing, China) and measured using ImageJ software (version 1.8.0, National Institutes of Health, Bonn, Germany).

TUNEL Staining

Tissue cryosections were subjected to Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) staining (C1090, Beyotime, Shanghai, China). The segments were immersed in 4% paraformaldehyde for 30 minutes. Proteinase K was used for a 20-minute reaction at 37 °C. The Terminal deoxynucleotidyl transferase (TdT) enzyme reaction mixture was kept in the dark at 37 °C for 60 minutes. The sections were then mounted with an anti-fade reagent mounting medium and observed and photographed under a fluorescence microscope (Olympus X71, Olympus, Tokyo, Japan).

Statistical Analysis

The analysis of the data was conducted using the statistical software SPSS 19.0 (International Business Machines Corporation, Armonk, NY, USA). The mean ± standard deviation was used to represent continuous data. Between-group comparisons were made using *t*-tests. One-way analysis of variance (ANOVA) was used to perform multiple-group mean comparisons, followed by Tukey's post hoc test. A *p*-value below 0.05 was deemed as statistical significance.

Results

CBD-BDNF Improves Functional Outcomes after SAH

Neurological deficit, balance beam, and Y-maze tests are common experimental methods for assessing animal behavioral and cognitive performance. In this study, they were used to evaluate the neurofunctional and cognitive

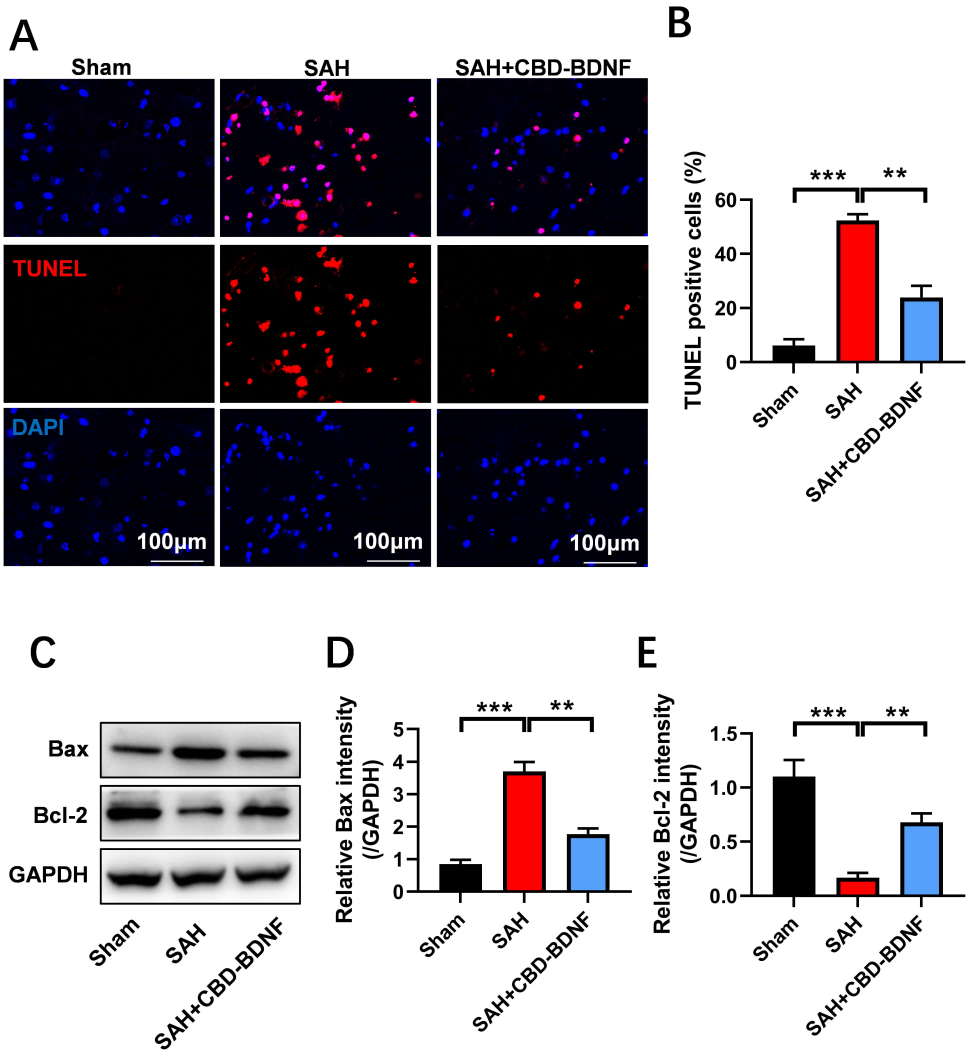

Fig. 4. CBD-BDNF alleviates neuronal apoptosis following subarachnoid hemorrhage. (A,B) Representative images of TUNEL staining (A) and quantitative analysis of TUNEL-positive cells (B). (C–E) Protein expression of Bax and Bcl-2 in the ipsilateral cortex detected by immunoblotting. $^{**}p < 0.01$, $^{***}p < 0.001$. n = 3. Scale bar = 100 μm. TUNEL, Terminal deoxynucleotidyl transferase dUTP Nick End Labeling.

performance of animals after experimental SAH and the impact of CBD-BDNF on these outcomes. Compared to the SAH model group, the CBD-BDNF treatment group exhibited better therapeutic outcomes in neurological deficit, balance beam, and Y-maze tests (Fig. 1A–C, $p < 0.01$). This indicates a significant positive impact of CBD-BDNF treatment on improving neurofunctional deficits, balance ability, and spatial working memory after SAH.

CBD-BDNF Reduces Inflammatory Response

IL-1β, IL-6, and IL-18 are inflammatory cytokines that play important roles in the inflammatory response and immune reactions and were quantitatively analyzed using ELISA kits. The Sham group had the lowest levels of inflammatory factors, indicating no significant inflammatory response. The SAH group had the highest levels of inflammatory factors, reflecting a strong inflammatory response induced by SAH (Fig. 2A–C, $p < 0.01$). However, the levels of inflammatory factors decreased in the group receiving CBD-BDNF treatment after SAH, suggesting that CBD-BDNF may help alleviate the inflammation caused by SAH (Fig. 2A–C, $p < 0.01$).

CBD-BDNF Inhibits the Activation of NLRP3 Inflammasome and Promotes the Expression of SIRT1

NLRP3 is an inflammasome-related protein whose overactivation is associated with inflammatory response and cell damage. In the experiment, the protein expression of NLRP3 significantly increased in the SAH group, while it decreased in the SAH+CBD-BDNF group (Fig. 3A, $p < 0.01$, and $p < 0.001$). This suggests that CBD-BDNF may alleviate neural inflammation by inhibiting the activation of NLRP3. ASC is a key protein in the assembly

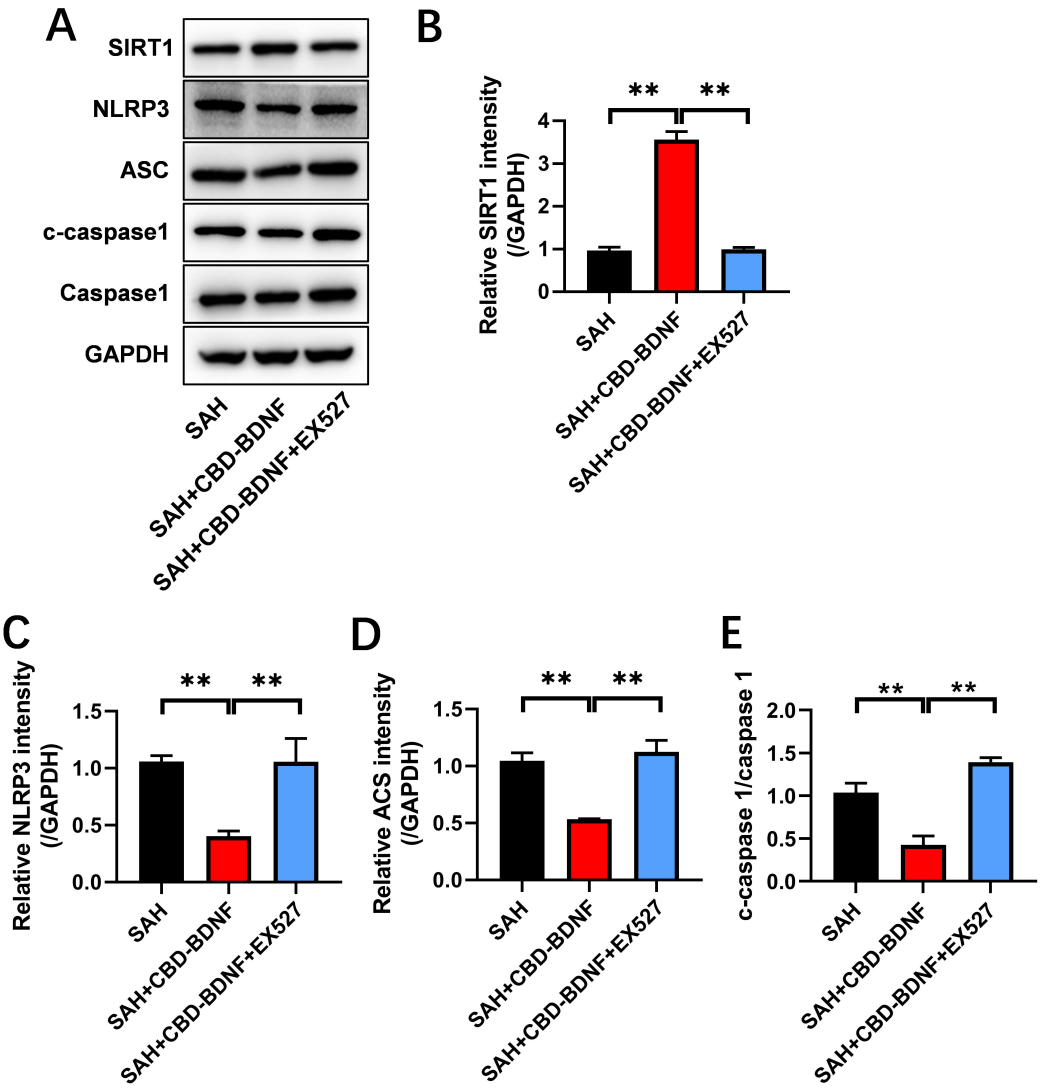

Fig. 5. SIRT1 inhibitor EX527 reverses the neuroprotective effect of CBD-BDNF. (A) Immunoblotting detected Protein expression of SIRT1, NLRP3, ASC, caspase-1, and c-caspase-1 in the ipsilateral cortex. (B–E) Quantitative analysis of SIRT1 (B), NLRP3 (C), ASC (D), c-caspase-1 (E) levels. $^{**}p < 0.01$, n = 3.

and activation process of the inflammasome. The protein ASC binds to the precursor of caspase-1 to form a multi-protein complex, which ruptures the cell and releases the contents, causing an inflammatory response. ASC significantly increased in the SAH group while it decreased in the SAH+CBD-BDNF group, which is consistent with the trend of NLRP3 changes (Fig. 3C, $p < 0.01$, and $p < 0.001$). The expression of c-caspase-1 increased in the SAH group, while it decreased in the SAH+CBD-BDNF group, supporting the inhibitory effect of CBD-BDNF on inflammasome activation (Fig. 3D, $p < 0.01$, and $p < 0.001$). SIRT1 is a deacetylase with anti-inflammatory and cell-protective effects. The experimental results show a significant increase in the expression of SIRT1 in the SAH+CBD-BDNF group (Fig. 3A–E, $p < 0.01$, and $p < 0.001$).

CBD-BDNF Alleviates Neuronal Apoptosis after SAH

The results of TUNEL staining show a significant increase in neuronal apoptosis in the SAH group compared to the Sham group. However, in the SAH+CBD-BDNF group, neuronal apoptosis is significantly reduced (Fig. 4A,B, $p < 0.05$). This suggests that CBD-BDNF can alleviate neuronal apoptosis after SAH. Bax and Bcl-2 are proteins related to cell apoptosis, with Bax promoting apoptosis and Bcl-2 inhibiting it. In the experiment, the expression of Bax increased in the SAH group while it decreased in the SAH+CBD-BDNF group, and the expression of Bcl-2 decreased in the SAH group while it increased in the SAH+CBD-BDNF group (Fig. 4C–E, $p < 0.01$, and $p < 0.001$). This indicates that CBD-BDNF may alleviate neuronal apoptosis after SAH by regulating the expression of Bax and Bcl-2. The experimental results show that CBD-

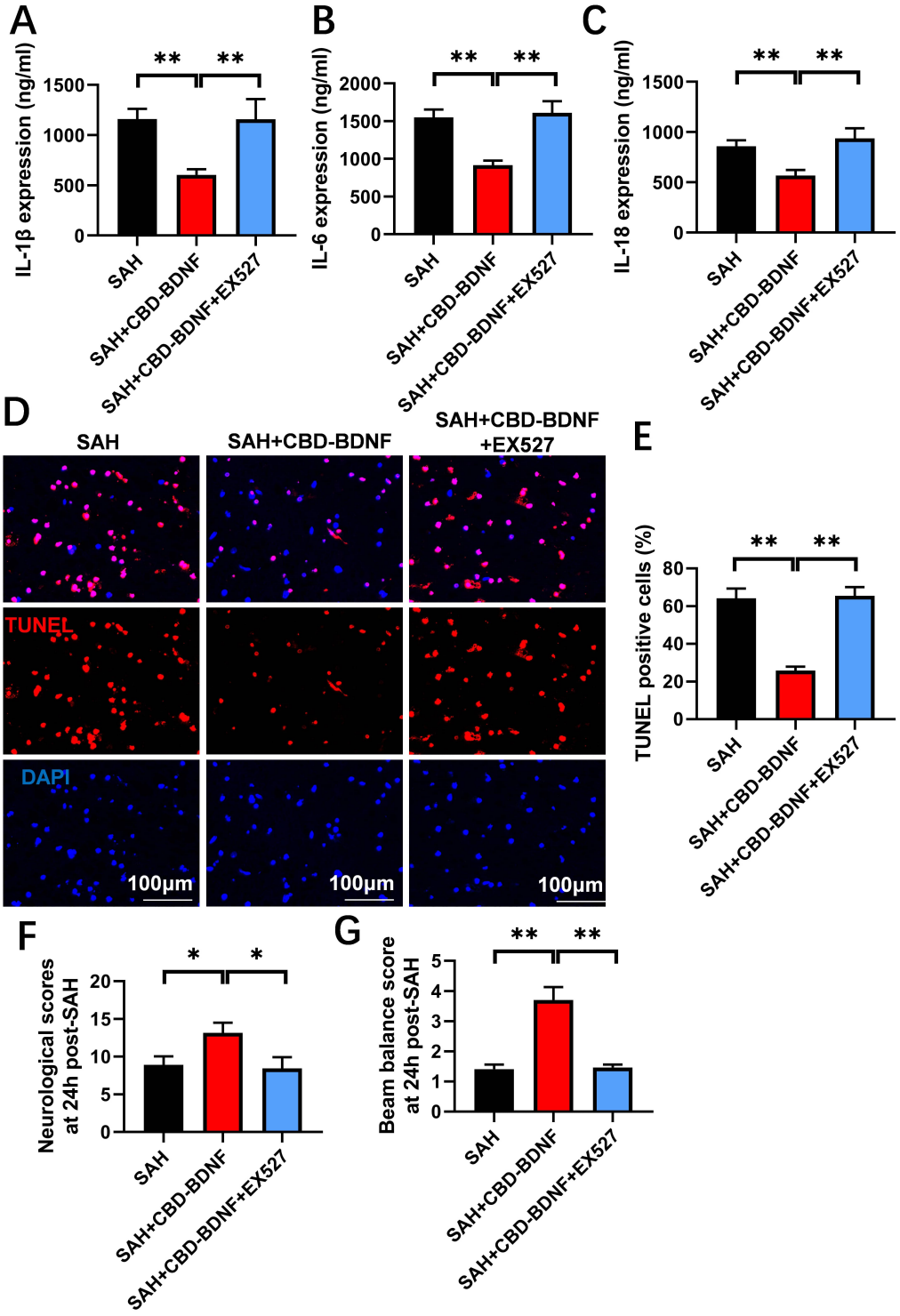

Fig. 6. Effects of EX527 on inflammation, apoptosis, and animal function. (A–C) Quantitative analysis of IL-1β (A), IL-6 (B), and IL-18 (C) levels using ELISA assay kits. (D,E) Representative images of TUNEL staining and quantification of TUNEL-positive cells. (F) Neurological deficit testing on the first day after subarachnoid hemorrhage. (G) Balance beam testing on the first day after subarachnoid hemorrhage. *$p < 0.05$, **$p < 0.01$, n = 3. Scale bar = 100 μm.

BDNF can improve experimental SAH-induced neural inflammation and neuronal damage by alleviating neuronal apoptosis.

The SIRT1 Inhibitor EX527 Reverses the Neuroprotective Effect of CBD-BDNF

SIRT1 exerts anti-inflammatory and cell-protective effects through acetylation. In the experiment, the expres-

sion of SIRT1 significantly increased in the SAH+CBD-BDNF group, while it decreased in the SAH+CBD-BDNF+EX527 group. This suggests that CBD-BDNF may exert its neuroprotective effect by promoting the expression of SIRT1, and EX527 may reverse this effect. NLRP3 is an inflammasome-associated protein, and ASC is involved in inflammasome assembly and activation. The protein ASC binds to the precursor of caspase-1 to form a multiprotein complex, which ruptures the cell and releases the contents, causing an inflammatory response. In addition, the protein levels of NLRP3, ASC, c-caspase-1, and caspase-1 were significantly downregulated in the SAH+CBD-BDNF group, while they were upregulated in the SAH+CBD-BDNF+EX527 group (Fig. 5A–E, $p < 0.01$). This indicates that CBD-BDNF may exert its neuroprotective effect by blocking the NLRP3 inflammasome pathway, and EX527 may reverse this effect. The reversal of the neuroprotective effect of CBD-BDNF by the SIRT1 inhibitor EX527 may be mediated through its impact on the expression of SIRT1 and the NLRP3 inflammasome pathway, regulating neural inflammation and neuronal damage.

The Impact of EX527 on Inflammatory Response, Cell Apoptosis, and Animal Function

Compared to the SAH group, the levels of the inflammatory factors IL-1β, IL-6, and IL-18 were significantly reduced in the SAH+CBD-BDNF group, while they increased in the SAH+CBD-BDNF+EX527 group (Fig. 6A–C, $p < 0.01$). This indicates that CBD-BDNF may suppress the inflammatory response by reducing the levels of inflammatory factors, and EX527 may reverse this effect. Compared to the SAH group, the number of apoptotic cells significantly decreased in the SAH+CBD-BDNF group and increased in the SAH+CBD-BDNF+EX527 group (Fig. 6D,E, $p < 0.01$). This suggests that CBD-BDNF may protect neurons by reducing cell apoptosis, and EX527 may reverse this effect. Neurological function deficit and beam balance testing assessed neurological function and spatial memory. The results show that in the SAH+CBD-BDNF group, the neurological function score and spatial memory significantly improved, while they decreased in the SAH+CBD-BDNF+EX527 group (Fig. 6F,G, $p < 0.05$, and $p < 0.01$). This indicates that CBD-BDNF may promote neural repair by improving neurological function and spatial memory, and EX527 may reverse this effect.

Discussion

Our findings indicate that CBD-BDNF significantly alleviates post-SAH neuroinflammation. The inflammatory response is a key driver of neurofunctional impairment and cell death following SAH [23,24]. Activation of the NLRP3 inflammasome plays a crucial role in this process [11]. Evidence from animal models and clinical studies suggests that the NLRP3 inflammasome is a major contributor to microglia-mediated inflammatory processes after SAH [25,26] and activates caspase-1, thereby promoting the maturation and secretion of the inflammatory cytokines IL-1β and IL-18 following brain injury [27,28]. The aptamer protein ASC is one of the main components of NLRP3 [26]. Our study demonstrates that in the animal models treated with CBD-BDNF, the expression levels and activity of NLRP3 and ASC inflammasome significantly decrease, accompanied by reduced levels of IL-1β and IL-18, indicating that CBD-BDNF effectively blocks the inflammatory response triggered by SAH. SIRT1, a Nicotinamide Adenine Dinucleotide (NAD)-dependent deacetylase, is involved in various intracellular processes, including anti-inflammatory, antioxidant, and cellular metabolic regulation [29–31]. Through deacetylating transcription factors such as NF-κB, SIRT1 can inhibit the expression of inflammatory genes [32]. Interestingly, we previously demonstrated that SIRT1 inhibits NLRP3 inflammasome signaling to improve EBI after SAH [33]. In our experiments, CBD-BDNF treatment increased the activity of SIRT1, reduced the activity of inflammatory factors, and decreased the expression of inflammatory cytokines. These results suggest that CBD-BDNF exerts its anti-inflammatory effects by activating SIRT1.

The neuroprotective effects of CBD-BDNF were also confirmed in our study. BDNF is a crucial neurotrophic factor for neuronal survival and repair [34,35]. Our experimental results show that post-treatment, CBD-BDNF significantly increases the survival rate of damaged neurons and reduces cell apoptosis. Mechanistically, this may be due to BDNF activating its receptor TrkB, which further activates signaling pathways for neuroprotection and cell survival, such as PI3K/Akt and MAPK/ERK.

This study revealed the potential inhibitory effect of CBD-BDNF on the NLRP3 inflammasome in an SAH model, providing a new target for treating inflammatory neural injury. By demonstrating the ability of CBD-BDNF to enhance SIRT1 activity, this study provides a new theoretical basis and experimental evidence for investigating the role of SIRT1 in SAH treatment. The study also highlights the potential of CBD-BDNF in improving post-SAH neurofunction and reducing neuronal damage, laying the foundation for future clinical translational research.

The study has limitations, primarily conducted in animal models. Although the model has been widely accepted and used, the clinical relevance of the results needs further validation in human patients. While CBD-BDNF has shown regulatory effects on NLRP3 inflammasome and SIRT1, the specific molecular mechanisms and signaling pathways are not fully elucidated, requiring further in-depth exploration in future research. For example, whether CBD-BDNF can further activate signaling pathways for neuroprotection and cell survival such as PI3K/Akt and MAPK/ERK, needs further investigation. Additionally, the study did not extensively examine the effects of CBD-

BDNF at different time points and doses, and future research should assess its dose-response relationship and optimal treatment window.

Conclusion

Our study provides new insights into the treatment of SAH and lays the foundation for further clinical research. The multiple mechanisms of action of CBD-BDNF, particularly its potential benefits in inhibiting neural inflammation and protecting neurons, offer a new direction for the treatment of SAH. Future research should focus on validating these findings and exploring the potential of CBD-BDNF in clinical applications.

Availability of Data and Materials

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author Contributions

The idea or design of the study: XSL and YWS. Analyzing data and drafting the research paper: YYS and YHX. Analysis and interpretation of data: SCG and CL. All authors have involved in drafting or critical revision of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experimental procedures used in the mice were approved by the Ethics Committee of Laoting County Hospital (Approval No.: LT2018-(C57)-01).

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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