Article

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Berberine Alleviates Sevoflurane Anesthesia-Induced Cognitive Dysfunction in Neonatal Mice via Regulating CREB1

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Background: Sevoflurane has been shown to stimulate neurotoxicity and lead to cognitive impairment. Berberine is known for its role in regulating nervous system diseases, including cognitive dysfunction. This study aimed to investigate the effects of berberine on cognitive dysfunction induced by sevoflurane anesthesia and its potential mechanisms.

Methods: In the *in vivo* study, neonatal mice were subjected to sevoflurane anesthesia to induce cognitive dysfunction. The cognitive function of the neonatal mice was evaluated using the Morris water maze test, open field test, and tail suspension test. Enzyme-linked immunosorbent assay (ELISA) was utilized to assess the levels of inflammatory factors. Immunohistochemistry (IHC) was conducted to detect ionized calcium-binding adaptor molecule 1 (IBA-1)-positive cells and cleaved caspase-3-positive cells in the hippocampus of the neonatal mice. Western blotting was used to measure the levels of cyclic adenosine monophosphate (cAMP) response element-binding protein 1 (CREB1) in hippocampal tissues and neurons. Hippocampal neurons were isolated from the hippocampus of neonatal mice. These neurons were treated with berberine or subjected to cell transfection. The cell counting kit-8 (CCK-8) assay and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay were conducted to measure cell viability and apoptosis of hippocampal neurons *in vitro*.

Results: Berberine significantly attenuated sevoflurane-induced cognitive impairment and inflammation in neonatal mice ($p < 0.05$ or $p < 0.01$). Additionally, berberine reduced sevoflurane-triggered neuronal apoptosis in the hippocampus of neonatal mice ($p < 0.01$). Sevoflurane markedly decreased CREB1 expression in the hippocampus of neonatal mice ($p < 0.01$), which was elevated by berberine treatment ($p < 0.01$). Mechanistically, sevoflurane significantly suppressed cell viability and promoted cell apoptosis of hippocampal neurons ($p < 0.0001$ or $p < 0.01$), which were mitigated by berberine ($p < 0.05$, $p < 0.01$, or $p < 0.001$). Furthermore, berberine significantly elevated CREB1 expression in sevoflurane-treated hippocampal neurons ($p < 0.01$). The beneficial effects of berberine on cell viability and apoptosis in sevoflurane-treated hippocampal neurons were blocked by CREB1 depletion ($p < 0.001$).

Conclusion: Our results demonstrated that CREB1 was significantly decreased in the hippocampus of sevoflurane-treated neonatal mice *in vivo* and in sevoflurane-treated hippocampal neurons *in vitro*. This decrease was mitigated by berberine treatment. Moreover, berberine improved sevoflurane anesthesia-induced cognitive impairment in neonatal mice by attenuating neuronal inflammation and apoptosis *in vivo*. The inhibitory effects of berberine on sevoflurane-induced cell apoptosis were reversed by CREB1 downregulation. These findings indicate that berberine protects against sevoflurane anesthesia-induced cognitive impairment by reducing apoptosis of hippocampal neurons, partially through increasing CREB1 expression.

Keywords: sevoflurane; cognitive impairment; berberine; CREB1; apoptosis

Introduction

Reports have indicated that some patients who underwent anesthesia and surgery early in life might be at risk for abnormal learning, behavior, and memory [1]. Similarly, preclinical research has observed learning and memory impairment, as well as abnormal behavior in neonatal animals post-surgery and anesthesia [2]. Accumulating evidence suggests that early general anesthesia can harm the development of the nervous system by repressing neurogenesis and impairing neurocognitive function [3,4]. Previous studies have reported that exposure to general anesthetics in young children may result in potential neurocognitive damage [5–7]. In recent years, the adverse effects of anesthesia on the nervous system have been widely recognized.

Sevoflurane, a commonly used clinical anesthetic, has been shown to trigger neuroinflammation and neuronal cell death in the hippocampus, affecting cognitive function after surgery through unclear mechanisms [8,9]. Neuroinflammation has been linked to the pathogenesis of sevoflurane-induced cognitive dysfunction [10], with elevated production of proinflammatory cytokines observed in mice exposed to sevoflurane [11]. Additionally, sevoflurane has been proven to induce apoptosis in hippocampal neurons, thereby causing neuronal damage in mice [12]. Thus, it is significant to explore potential protective treatment targets for sevoflurane-induced neuronal damage and cognitive impairment in newborns.

Berberine is a natural alkaloid derived from traditional Chinese medicines, such as *cortex phellodendri* (Huangbai) and *coptis chinensis* (Huanglian). It has a variety of pharmacological effects, including hypoglycemic action, antitumor, anti-inflammatory, anti-bacterial, anti-viral, lipid regulation, and anti-depression [13]. Several clinical trials have provided evidence that berberine can be used for treating schizophrenic patients by preventing metabolic disturbances [14,15]. Berberine has also shown positive effects on depressive behavior in animal models [16]. The neuroprotective effects of berberine have been demonstrated in neurodegenerative disorders [17,18]. Regarding cognitive impairment-related diseases, berberine has been shown to improve diabetes-induced cognitive impairments through its neuroprotective function in *in vivo* animal studies [19]. Additionally, the protective effects of berberine on cognitive disorders have been verified in animal models of Alzheimer's disease [20–22]. However, the molecular mechanisms of berberine in sevoflurane-induced cognitive impairment remain unclear.

Cyclic adenosine monophosphate (cAMP) response element-binding protein 1 (CREB1) is a member of the basic-region leucine zipper superfamily [23]. As a transcriptional activator, CREB1 facilitates neuronal plasticity and the formation of long-term memory, and it plays a role in modulating synaptic plasticity [24,25]. Moreover, CREB1 is well-known for its role in neurons, playing a vital role in neuronal survival by activating the expression of several genes [26,27]. A report revealed that dysregulated genes related to CREB1 were observed in the brains of Alzheimer's disease patients, evidenced by decreased expression of brain-derived neurotrophic factor regulated by CREB1 [28–30]. Additionally, CREB1 is involved in regulating neuronal injury and cognitive dysfunction in vascular dementia rats [31]. However, whether berberine can affect sevoflurane-induced cognitive impairment in neonatal mice by modulating CREB1 remains to be investigated.

Our objective was to verify whether berberine ameliorates cognitive impairment caused by sevoflurane and to explore whether CREB1 mediates the protective effects of berberine on neurons in neonatal mice.

Materials and Methods

Animals and Drugs Treatment

A total of 48 male and female C57BL/6 mice, aged 7 days and weighing between 10 and 15 grams, were utilized in the present study due to their heightened susceptibility to neuronal insult induced by general anesthetics [32]. The mice were procured from Beijing Laboratory Animal Technology Co., Ltd. (Beijing, China) and were housed under controlled conditions: a 12-hour light/12-hour dark cycle, room temperature maintained at 23 $\pm$ 1 °C, and humidity at 60 $\pm$ 5%. All animal experimental procedures were ethically approved by the Beijing Biocisco Biomedical Technology Co., Ltd. Ethical Committee (No. MDL2023-05-25-04). Berberine (purity: 98%; YM-YW1079) was acquired from Shanghai Yuanmu Bio-Technology Co., Ltd. (Shanghai, China).

All 48 mice were randomly assigned to three groups, each comprising 16 individuals: the Control group (n = 16), the sevoflurane group (Sevo; n = 16), and the sevoflurane+berberine group (Sevo+BBR; n = 16). Mice in the Control group were exposed to 40% oxygen, while those in the Sevo and Sevo+BBR groups were exposed to 2.2% sevoflurane for 2 hours [12]. Following sevoflurane anesthesia, mice in the Sevo+BBR group received berberine (100 mg/kg) for 7 days, as previously described [33]. Subsequently, 10 mice from each group were euthanized via intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight) and perfused transcardially with saline. Their hippocampi were promptly dissected out for immunohistochemistry (IHC; n = 5), enzyme-linked immunosorbent assay (ELISA), and western blotting analysis (n = 5). The remaining mice (n = 6) in each group underwent the Morris water maze test, open field test, and tail suspension test before being euthanized by cervical dislocation.

Open Field Test

A plain open field arena measuring 44 cm $\times$ 44 cm $\times$ 30 cm was utilized to assess anxiety-like behavior and locomotor activity [34]. After a 30-second habituation period,

the total distances traveled and time spent in the 14.7 cm × 14.7 cm center arena were recorded during each 5 min session.

Tail Suspension Test

Mice were suspended by their tails using adhesive tape, positioned 1 cm from the tail tip and suspended 15 cm above the ground. Small plastic tubes were affixed to the tails of the mice to prevent climbing. The tail suspension test lasted for 6 minutes, during which the immobility time was assessed during the final 4 minutes.

Morris Water Maze Test

The Morris water maze test was conducted following previously established protocols [35]. A circular tank measuring 120 cm in diameter and 50 cm in height contained a submerged platform with a diameter of 10 cm. Training sessions were administered twice daily over a period of 4 days. Mice were placed in various quadrants of the tank and given 60 seconds to freely locate the hidden platform in the water. Upon locating the platform, mice remained on it for 15 seconds. If a mouse failed to find the platform within 60 seconds, it was guided to the platform and allowed to stay on it for 15 seconds. The latency, or time taken to find the platform, was recorded.

A probe trial was conducted without the platform to assess memory consolidation. Mice were placed in different quadrants, and the platform was removed from the pool. They were then given 60 seconds to swim freely, during which the frequency of platform crossings was recorded.

ELISA

The levels of tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and interleukin-6 (IL-6) in the hippocampus samples were measured using mouse TNF-α (MTA00B-1), IL-1β (MLB00C-1), and IL-6 (M6000B-1) ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA). The assays were performed according to the manufacturer's instructions.

IHC Assay

The hippocampal tissue was deparaffinized and rehydrated. Slides were then treated with 3% (v/v) hydrogen peroxide and subjected to antigen retrieval using citrate at high temperature. Next, the slides were incubated overnight at 4 °C with anti-ionised calcium-binding adaptor molecule 1 (IBA-1) (ab178846; 1:2000, Abcam, Cambridge, MA, USA) and anti-cleaved caspase-3 (PA5-114687; 1:200, Thermo Fisher, Waltham, MA, USA) antibodies, followed by incubation with the secondary antibody (ab205718; 1:10000, Abcam, Cambridge, MA, USA). Positive cells were visualized and imaged using a microscope (ZEISS Axioscope 5; Carl Zeiss AG, Heidenheim, Germany).

Western Blotting

Total proteins were extracted using radioimmunoprecipitation assay (RIPA) buffer (20-188; Millipore, Bradford, MA, USA), and the protein samples were separated by electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gel for 2 hours. Subsequently, the proteins were transferred onto polyvinylidene fluoride membranes (03010040001; Roche, Basel, Switzerland). Blocking of non-specific antigens was achieved with 5% skim milk before incubation with primary antibodies, including anti-cleaved caspase-3 (ab32042; 1:1000, Abcam, Cambridge, MA, USA), anti-CREB1 (ab32515; 1:1000, Abcam, Cambridge, MA, USA), and anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH; ab8245; 1:1000, Abcam, Cambridge, MA, USA) overnight at 4 °C. Following incubation with the secondary antibody (ab205718; 1:20000, Abcam, Cambridge, MA, USA) for 1.5 hours, the protein bands were visualized using an enhanced chemiluminescence (ECL) kit (WBULP-100ML; Millipore, Bradford, MA, USA) and analyzed using ImageJ software (Version 1.52a, NIH, Bethesda, MD, USA).

Isolation of Primary Hippocampal Neurons and Cell Treatment

The extracted hippocampal tissue was sliced into 4 µm-thick sections and treated with 0.125% trypsin. Following trituration and centrifugation, cells at a density of 1×10^6 cells/mL were plated onto poly-D-lysine (10 mM)-coated dishes in Neurobasal medium (21103049; Invitrogen, Carlsbad, CA, USA) supplemented with 0.25% Glumax and 2% B27 (12587-010; Invitrogen, Carlsbad, CA, USA). After 3 days, the cells were further cultured with cytosine arabinoside (2.5 µg/mL) for an additional 24 hours. Fourteen days later, the primary hippocampal neurons were harvested and utilized for functional assays. These neurons were stained positive for NeuN and tested negative for mycoplasma.

The isolated primary hippocampal neurons were cultured in Neurobasal medium containing 2.78 mM glucose and 1% B27 under 2.2% sevoflurane in an anesthesia machine for 6 hours. Various concentrations (5, 10, and 20 µM) of berberine were then used to treat the primary hippocampal neurons for 24 hours.

Immunofluorescence Staining

Primary hippocampal neurons were identified using NeuN immunofluorescence staining. Initially, cells were fixed with 4% paraformaldehyde (Beyotime, China) for 15 minutes, followed by permeabilization with 0.1% Triton X-100 (Beyotime, Shanghai, China). Subsequently, cells were incubated with the primary antibody NeuN (ab177487, 1:100; Abcam, Cambridge, MA, USA) overnight at 4 °C. After washing with PBS, cells were then incubated with TRITC-conjugated Goat Anti-Rabbit IgG H&L (ab6718, 1:1000; Abcam, Cambridge, MA, USA)

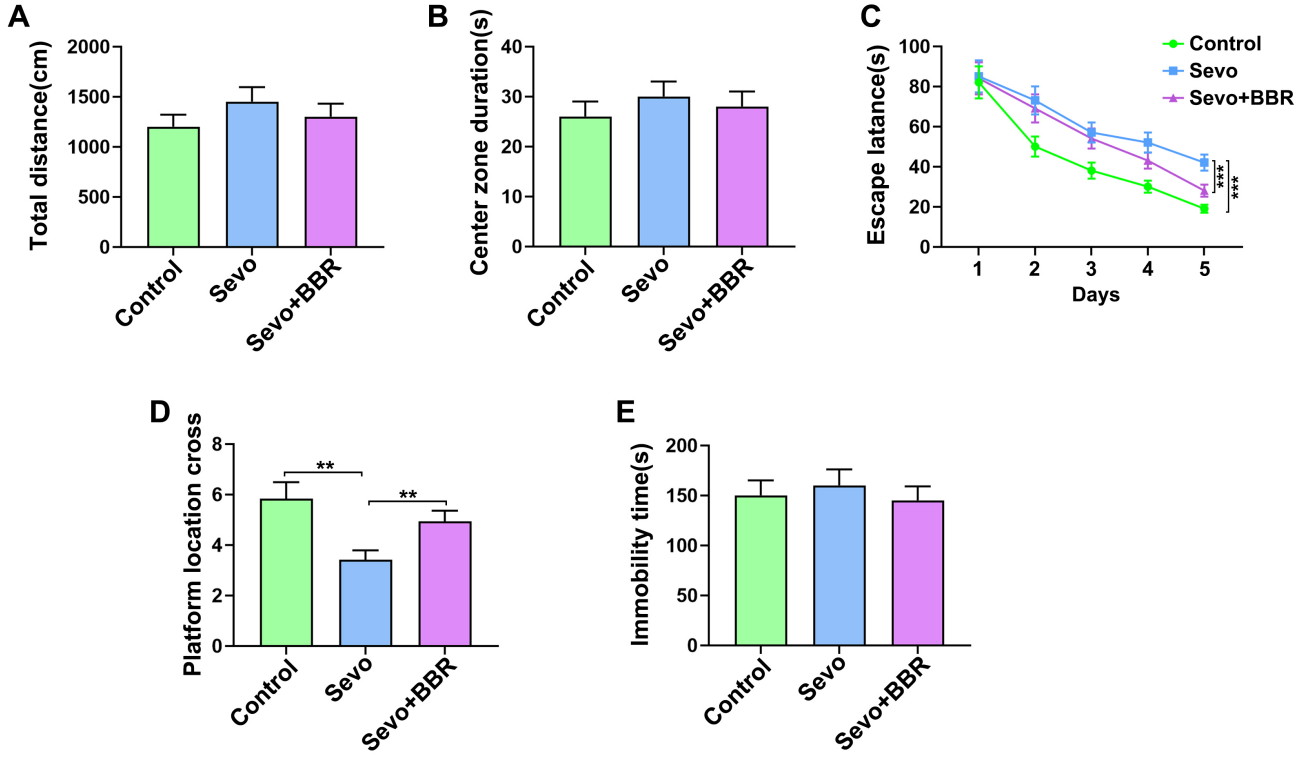

Fig. 1. Berberine improved sevoflurane-caused cognitive dysregulation in neonatal mice. (A) The total distance traveled by each group was recorded in the open field test (n = 6). (B) The duration of mice staying in the central area was recorded in the open field test (n = 6). (C,D) Morris water maze test was conducted, and escape latency and platform crossing frequency were recorded to evaluate memory and spatial learning functions in neonatal mice (n = 6). (E) Immobility time of the mice was recorded in the tail suspension test (n = 6). $^{**}p < 0.01$, and $^{***}p < 0.001$. Sevo, sevoflurane; BBR, berberine.

at room temperature for 1 hour. Finally, the cells were stained with 4′,6-diamidino-2-phenylindole (DAPI; Beyotime, Shanghai, China) under dark conditions for 10 minutes, and cell images were captured using an optical microscope (ZEISS Axioscope 5; Carl Zeiss AG, Heidenheim, Germany).

Cell Transfection

SiRNA targeting *CREB1* (si-*CREB1*; 5′-GCAGCUCGAGAGUGUCGUATT-3′ (F), 5′-UACGACACUCUCGAGCUGCTT-3′ (R)) and the negative control (si-NC; 5′-UUCUCCGAACGUGUCACGUTT-3′ (F), 5′-ACGUGACACGUUCGGAGAATT-3′ (R)) were procured from Genepharma (Shanghai, China). Primary hippocampal neurons were transfected with the aforementioned oligonucleotides and plasmids using Lipofectamine 3000 reagent (L3000015, Invitrogen, Carlsbad, CA, USA).

Cell Viability and Apoptosis

Primary hippocampal neurons (2×10^4 cells) treated with the indicated concentrations of berberine and transfected with siRNA were incubated with 10 µL of cell counting kit-8 (CCK-8) reagent (CA1210; Solarbio, Beijing, China) for 4 hours. After washing with phosphate-buffered saline, the absorbance was measured at 450 nm using a microplate reader (Multiskan MK3; Thermo Labsystems, Waltham, MA, USA).

For cell apoptosis analysis, primary hippocampal neurons (3×10^5 cells) treated with the indicated concentrations of berberine and transfected with siRNA were cultured with terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) reaction buffer (E-EL-0003; Elabscience, Wuhan, China), 2% 3,3′-diaminobenzidine (DAB), and Converter-peroxidase. After counterstaining with hematoxylin, the cells were visualized using an optical microscope (ZEISS Axioscope 5; Carl Zeiss AG, Heidenheim, Germany), and the TUNEL-positive hippocampal neurons were counted.

Statistical Analysis

Statistical analysis of the data, presented as mean ± standard error of the mean, was performed using GraphPad Prism 6.01 (GraphPad Software, La Jolla, CA, USA). Differences between the two groups were assessed using the *t*-test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) was employed, followed by the Tukey test as a post-hoc analysis. A *p*-value less than 0.05 was considered statistically significant.

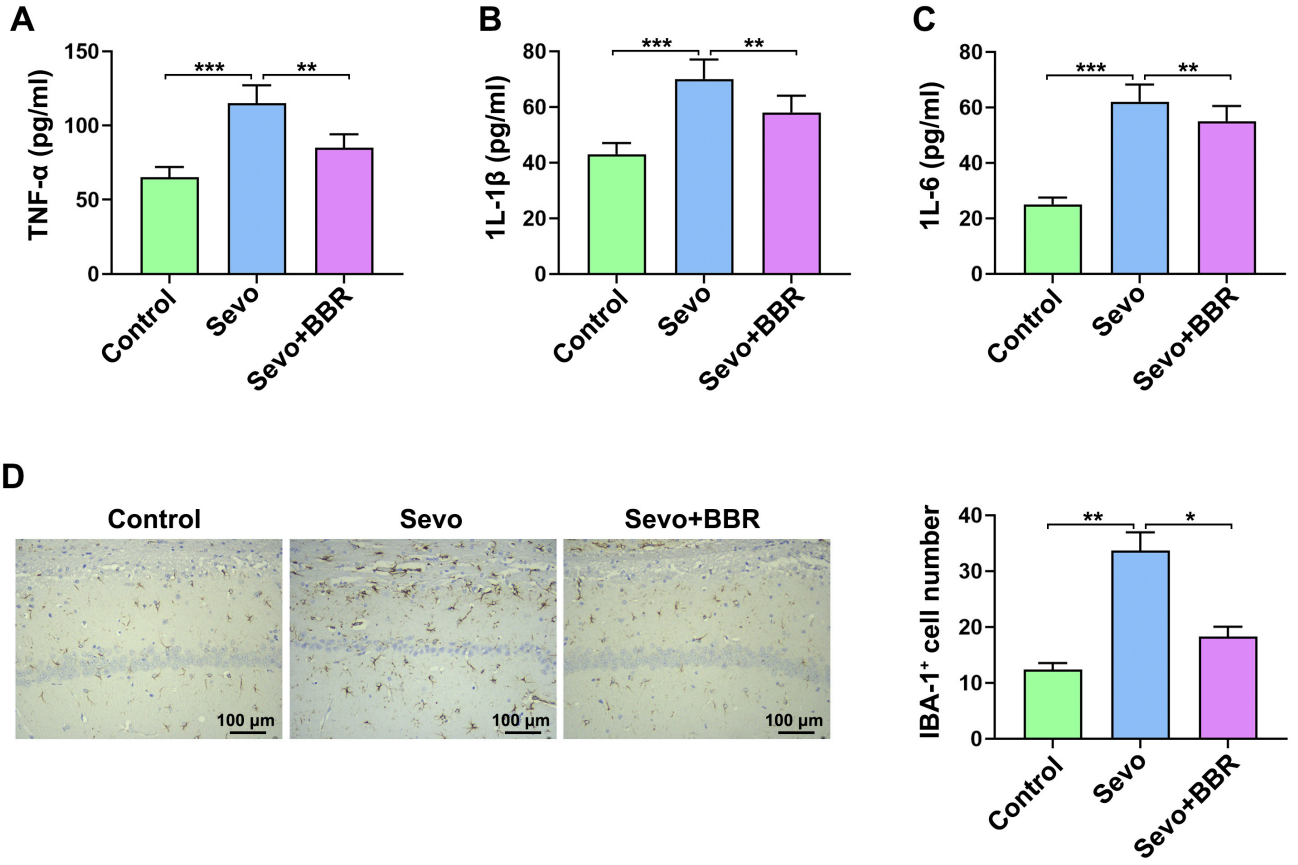

Fig. 2. Berberine alleviated sevoflurane-induced inflammation in neonatal mice. (A–C) ELISA kit was used to detect the levels of TNF-α, IL-1β and IL-6 in the hippocampus samples (n = 5). (D) IHC assay was conducted to detect the ration of IBA-1-positive cells in mice hippocampus sections (n = 5). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. ELISA, enzyme-linked immunosorbent assay; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1beta; IL-6, interleukin-6; IHC, immunohistochemistry; IBA-1, ionised calcium-binding adaptor molecule 1.

Results

Berberine Improved Sevoflurane-Caused Cognitive Dysregulation in Neonatal Mice

In the open field test, sevoflurane did not significantly alter the total distance traveled or the duration spent in the center area in neonatal mice ($p > 0.05$), indicating no discernible impact of sevoflurane on depressive/anxiety-like behavior in this population (Fig. 1A,B).

Results from the Morris water maze test revealed that sevoflurane substantially increased the escape latency of mice and decreased the frequency of platform crossings ($p < 0.01$ or $p < 0.001$). However, these effects were mitigated by berberine treatment ($p < 0.01$ or $p < 0.001$), suggesting that berberine administration could attenuate sevoflurane-induced cognitive impairment in neonatal mice (Fig. 1C,D).

In the tail suspension test, the immobility time remained unchanged following sevoflurane treatment ($p > 0.05$), indicating that sevoflurane did not induce a depressive state in neonatal mice (Fig. 1E).

Berberine Alleviated Sevoflurane-Induced Inflammation in Neonatal Mice

The ELISA results indicated a significant increase in the levels of TNF-α, IL-1β, and IL-6 in the hippocampus of neonatal mice exposed to sevoflurane ($p < 0.001$), which were suppressed by berberine treatment ($p < 0.01$) (Fig. 2A–C). To further investigate the suppressive effects of berberine on sevoflurane-induced inflammation, the activation of microglia in the hippocampus was examined using IHC. The data revealed a notable increase in IBA-1-positive cells, a marker for microglia, in response to sevoflurane exposure ($p < 0.01$). Furthermore, the activation of IBA-1 induced by sevoflurane was significantly attenuated by berberine treatment in neonatal mice ($p < 0.05$) (Fig. 2D).

Berberine Markedly Increased CREB1 Expression, and Alleviated Neuronal Apoptosis in the Hippocampus of Sevoflurane-Induced Neonatal Mice

Our data revealed a significant reduction in the protein expression of CREB1 in the hippocampus of neonatal mice treated with sevoflurane ($p < 0.01$), while its expression

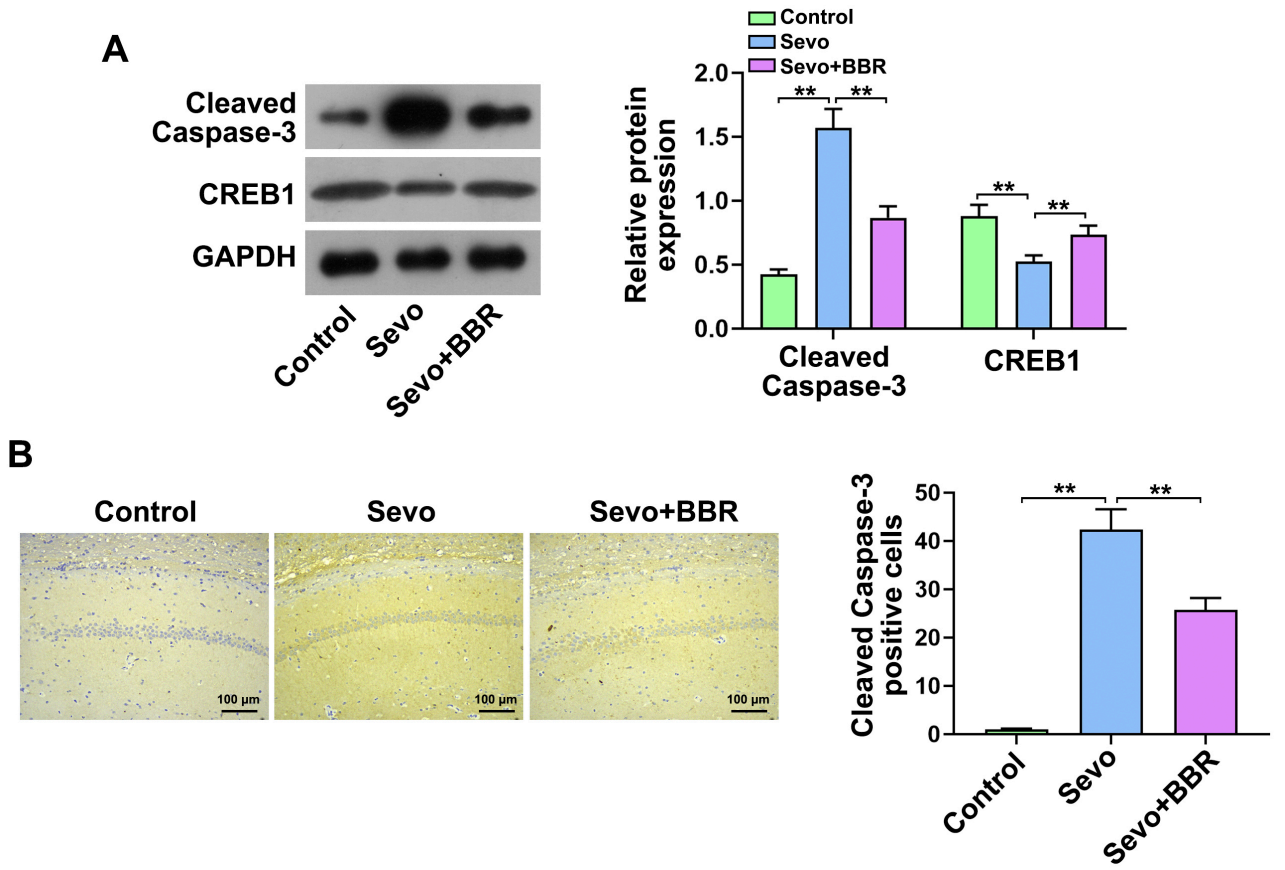

Fig. 3. Berberine markedly increased CREB1 expression, and alleviated neuronal apoptosis in the hippocampus of sevoflurane-induced neonatal mice. (A) Western blotting was used to assess the expression of CREB1, and apoptosis-related protein cleaved caspase-3 in the hippocampus of mice (n = 3). (B) The cleaved caspase-3 positive cells in the hippocampus of mice were determined using IHC assay (n = 5). $^{**}p < 0.01$. CREB1, cAMP response element binding protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

markedly increased after berberine treatment ($p < 0.01$) (Fig. 3A). Given the reported correlation between cognitive impairment induced by anesthesia and neuronal apoptosis, we further investigated the effects of berberine on neuronal apoptosis in sevoflurane-induced neonatal mice. Sevoflurane treatment significantly upregulated cleaved caspase-3 expression ($p < 0.01$), which was mitigated by berberine in the hippocampus of sevoflurane-treated neonatal mice ($p < 0.01$) (Fig. 3A). Similarly, the number of cleaved caspase-3 positive cells was significantly increased by sevoflurane treatment ($p < 0.01$), and this effect was inhibited by berberine in the hippocampus of neonatal mice ($p < 0.01$) (Fig. 3B).

Berberine Significantly Elevated CREB1 Expression, and Reduced Sevoflurane-Induced Apoptosis of Hippocampal Neurons in Vitro

Next, we investigated the effects of berberine on hippocampal neurons *in vitro*. NeuN immunofluorescence staining confirmed successful isolation of hippocampal neurons from mouse hippocampal tissue (Fig. 4A), revealing dendritic protrusions with a characteristic long slender appearance of neurons (Fig. 4A).

The CCK-8 assay demonstrated that different concentrations of berberine (5, 10, and 20 μM) significantly increased cell viability in sevoflurane-treated hippocampal neurons ($p < 0.05$ or $p < 0.001$) (Fig. 4B). Specifically, 5 μM of berberine exhibited the lowest promotion effect on cell viability compared to 10 and 20 μM of berberine in sevoflurane-treated hippocampal neurons. Moreover, 10 and 20 μM of berberine showed similar effects on cell viability, with no significant difference observed between the two groups. Thus, 10 μM of berberine was selected for subsequent experiments.

The TUNEL assay revealed that sevoflurane induced cell apoptosis ($p < 0.01$), while berberine markedly suppressed cell apoptosis in sevoflurane-treated hippocampal neurons ($p < 0.01$) (Fig. 4C). Additionally, the promotion effect of sevoflurane on cleaved caspase-3 expression was reversed by berberine treatment in hippocampal neurons *in vitro* ($p < 0.01$) (Fig. 4D). Moreover, the protein expression of CREB1 was reduced by sevoflurane ($p < 0.01$), which was rescued after berberine treatment in hippocampal neurons *in vitro* ($p < 0.01$) (Fig. 4D).

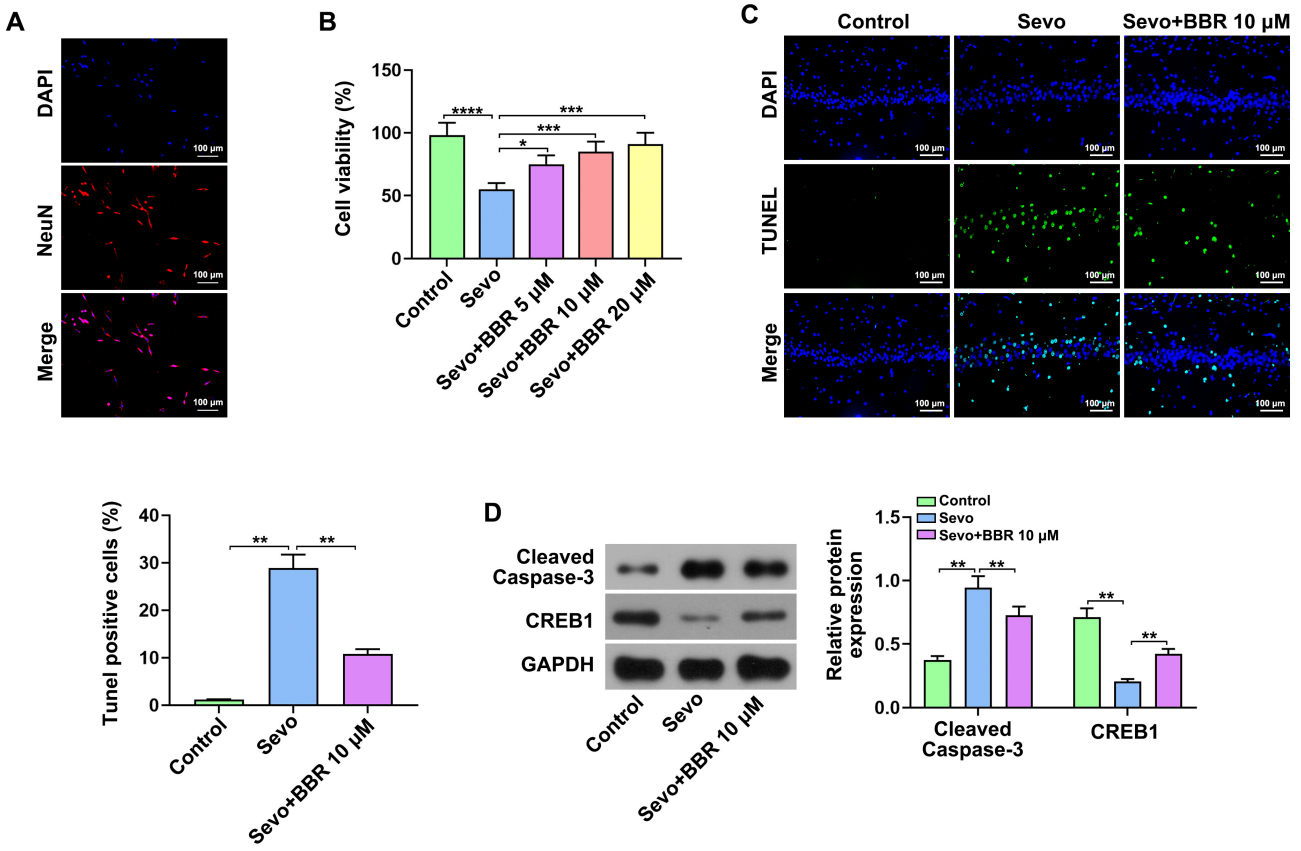

Fig. 4. Berberine significantly elevated CREB1 expression, and reduced sevoflurane-induced apoptosis of hippocampal neurons *in vitro*. (A) The hippocampal neurons were stained for NeuN. (B) The hippocampal neurons were exposed to sevoflurane for 6 hours, and then treated with different concentrations of berberine (5, 10 and 20 μM) for 24 hours, and cell viability was detected via CCK-8 assay (n = 3). (C) Hippocampal neurons were exposed to sevoflurane for 6 hours, and then treated with 10 μM of berberine for 24 hours; cell apoptosis was measured by TUNEL assay (n = 3). (D) Western blotting was utilized to determine the protein expression of cleaved caspase-3 and CREB1 in hippocampal neurons (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. CCK-8, cell counting kit-8; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; DAPI, 4′,6-diamidino-2-phenylindole.

Berberine Improved Sevoflurane-Induced Apoptosis of Hippocampal Neurons through Upregulating CREB1

The protein expression of CREB1 was significantly downregulated by CREB1 knockdown in hippocampal neurons ($p < 0.001$) (Fig. 5A). CREB1 knockdown attenuated the promotive effect of berberine on cell viability and the inhibitory effect on cell apoptosis in sevoflurane-treated hippocampal neurons ($p < 0.001$) (Fig. 5B–D). Moreover, the promotive effects of berberine on CREB1 expression in sevoflurane-treated hippocampal neurons were abrogated by CREB1 depletion ($p < 0.001$) (Fig. 5D).

Discussion

With the increased exposure of neonates to anesthetics during the development of the central nervous system, the relationship between brain damage and the adverse effects of anesthetic exposure has garnered more attention [36,37]. Studies have demonstrated that prolonged exposure to sevoflurane anesthesia inhibits neural stem cell self-renewal and promotes neuronal apoptosis [38,39]. The reduction in neuronal cells can result in deficits in learning and memory [40]. Therefore, investigating potential protective factors and the mechanisms underlying sevoflurane-induced cognitive impairment is warranted.

Sevoflurane anesthesia has been shown to induce neuronal cell damage, leading to deficits in learning and memory, as indicated by reduced platform crossing and increased escape latency [41]. Consistent with these findings, our results also demonstrate that sevoflurane treatment can impair cognitive function in neonatal mice, as evidenced by increased escape latency and decreased platform crossing frequency. The hippocampus plays a crucial role in memory, cognition, and navigation [42], and our data indicate that sevoflurane induces inflammation, as evidenced by elevated levels of inflammatory factors and increased IBA-1-positive cells in the hippocampus of neonatal mice. Sevoflurane treatment also induces hippocampal neuronal apoptosis in neonatal mice. Furthermore, sevoflurane in-

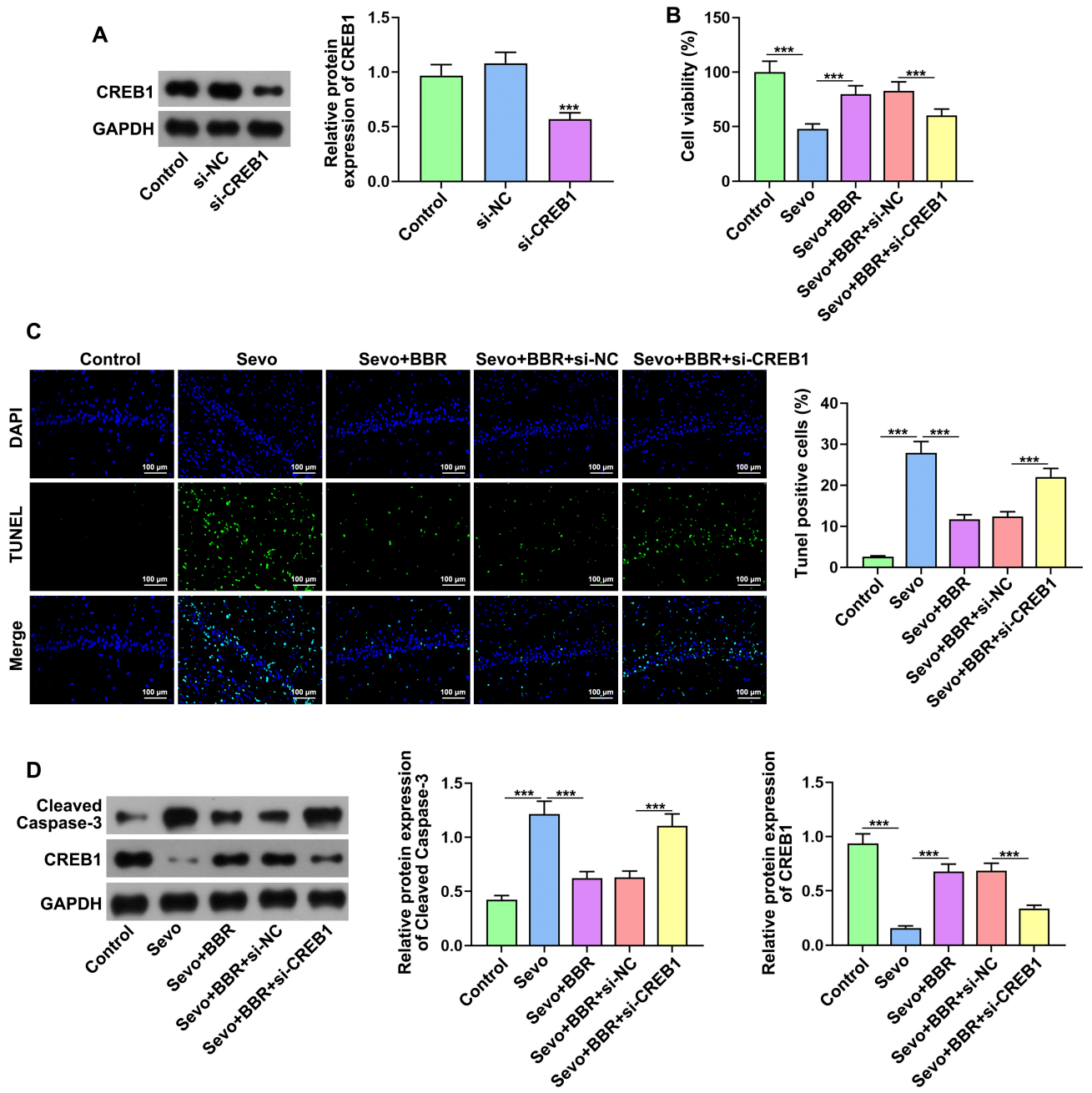

Fig. 5. Berberine improved sevoflurane-induced apoptosis of hippocampal neurons through upregulating CREB1. (A) The hippocampal neurons were transfected with si-NC and si-*CREB1*, and the protein expression of CREB1 was determined by western blotting (n = 3). (B–D) The hippocampal neurons were treated with sevoflurane, sevoflurane+berberine, sevoflurane+berberine+si-NC, and sevoflurane+berberine+si-*CREB1*. (B,C) CCK-8 and TUNEL assays were conducted to measure cell viability and apoptosis, respectively (n = 3). (D) Western blotting was applied for detecting cleaved caspase-3 and CREB1 expression (n = 3). ***$p < 0.001$. NC, negative control.

hibits cell viability and promotes apoptosis of primary hippocampal neurons *in vitro*. Overall, sevoflurane treatment in neonatal mice induces neuroinflammation and neuronal apoptosis, leading to cognitive impairment.

The current research focuses on elucidating the functional role of berberine in sevoflurane-treated neonatal mice and primary hippocampal neurons. Berberine, a natural alkaloid, has garnered significant attention in the study of central nervous system diseases [43]. It exhibits various neuroprotective properties by suppressing oxidation, neuroinflammation, and endoplasmic reticulum stress, thus demonstrating anti-inflammatory, antioxidant, and neuroprotective effects, ultimately attenuating neuronal damage and apoptosis [44]. Accumulating evidence highlights

the efficacy of berberine in conditions related to cognitive impairment-related conditions [19,45,46]. For instance, berberine alleviates cognitive defects in APP/PS1 mice by ameliorating endoplasmic reticulum stress [47].

Our findings reveal that berberine treatment protects against sevoflurane-triggered neurotoxicity and cognitive impairments in neonatal mice. Additionally, berberine alleviates the decrease in cell viability and increase in cell apoptosis of primary hippocampal neurons induced by sevoflurane treatment *in vitro*. These results suggest that berberine may improve sevoflurane-induced cognitive impairment by reducing hippocampal neuronal apoptosis.

The influence of the CREB family on neuronal system damage has been well-documented [48]. Members of the CREB family possess the ability to enhance neuronal survival, regulate synaptogenesis, modulate neuronal migration, and promote the formation of long-term memory and potentiation. Among these, CREB1 is particularly renowned for its role in neurons. A previous study has highlighted the crucial involvement of CREB1 in conditions such as epilepsy, where activated CREB1 expression plays a vital role [49]. Additionally, CREB1 expression regulated by Methyl CpG binding protein 2 (MECP2) has been implicated in age-related cognitive decline in mouse models [50]. Suppression of the CREB pathway has been shown to accelerate postoperative dysfunction of learning and memory in neonatal rat models [51].

In this study, our data demonstrate that CREB1 expression is decreased in the hippocampus of sevoflurane-treated neonatal mice *in vivo* and in sevoflurane-treated primary hippocampal neurons *in vitro*, with levels being restored following berberine treatment. Furthermore, the protective effects of berberine against sevoflurane-induced damage to primary hippocampal neurons were found to be attenuated by CREB1 knockdown, suggesting that berberine protects against sevoflurane-induced damage to primary hippocampal neurons by upregulating CREB1 expression *in vitro*.

It is noteworthy that study has reported gender differences in the effects of sevoflurane on the central nervous system of newborn mice [52], although contradictory results have also been reported [53]. This variability may be influenced by the birth day of the mice. Our study did not specifically differentiate between genders, which may introduce gender-related biases. Further research is warranted to elucidate any gender-related differences.

Conclusion

In summary, our study demonstrates that berberine can ameliorate cognitive impairment and increase CREB1 expression in neonatal mice exposed to sevoflurane. Moreover, berberine markedly attenuates sevoflurane-induced neuronal damage by not only enhancing cell viability but also reducing apoptosis in primary hippocampal neurons, through the upregulation of CREB1 *in vitro*. These findings hold potential clinical significance for neonates undergoing sevoflurane anesthesia.

Availability of Data and Materials

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

Author Contributions

LH and HW designed the research study and wrote the first draft. HW, WW and QZ performed the research. FY and HZ provided help and advice on the immunofluorescence staining. FY, HZ and GY analyzed the data. All authors contributed significantly to editorial changes of important content. 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

All animal experimental procedures were ethically approved by the Beijing Biocisco Biomedical Technology Co., Ltd. Ethical Committee (No. MDL2023-05-25-04).

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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