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Dexmedetomidine alleviates hippocampal neuronal damage in epilepsy through BDNF/TrkB pathway by inhibiting MeCP2

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Epilepsy (EP) is a prevalent neurological disorder. The study sought to investigate the impact of dexmedetomidine (DEX) on hippocampal neuron damage, methyl-CpG binding protein 2 (MeCP2) expression, and brain-derived neurotrophic factor (BDNF)/tyrosine receptor kinase B (TrkB) pathway in EP *in vitro* model. The study involved the isolation of hippocampal neurons from newborn neonatal rats, which were identified utilizing microscopic observation and immunofluorescence staining. The EP *in vitro* model was developed using magnesium-free treatment. Next, the neurons were treated with 0, 1, 10, 100, and 200 μM DEX to investigate its impact on EP. Neuron viability and apoptosis were assessed using CCK-8, western blotting, and TUNEL assay. The levels of IL-6 and TNF-α were measured using ELISA. The determination of ROS and MDA levels and SOD activity was conducted to evaluate oxidative stress. Moreover, the binding of MeCP2 to the BDNF promoter was confirmed using a ChIP assay. The hippocampal neurons were successfully extracted from newborn neonatal rats. DEX of 100 and 200 μM significantly promoted neuronal viability and inhibited neuronal apoptosis, inflammation, oxidative stress, and MeCP2 expression induced by magnesium-free. MeCP2 inhibited the expression of BDNF/TrkB pathway by binding to the BDNF promoter. Moreover, MeCP2 silencing promoted neuronal viability and inhibited apoptosis, inflammation, and oxidative stress, while BNDF silencing restored it. Furthermore, DEX alleviated hippocampal neuronal damage. However, MeCP2 overexpression restored it. DEX alleviated hippocampal neuronal damage in EP through BDNF/TrkB pathway by down-regulating MeCP2 expression. DEX might be one of novel and effective anti-seizure medications.

Keywords: BDNF/TrkB pathway, Dexmedetomidine, Epilepsy, Hippocampal neuron, MeCP2

Epilepsy (EP) is a chronic neurological disorder characterized by abnormal patterns of brain activity, known as seizures, which are often accompanied by symptoms such as convulsions, loss of consciousness, and mental absence[1,2]. The relationship between the causes, prognosis, and symptomatology of epilepsy is complex and not fully comprehended. It has been postulated that the epileptogenic process involves a positive feedback loop, in which seizures lead to further seizures through intricate interactions involving neuronal death, gliosis, emergence of abnormal connectivity, hyper-excitation, and neuroinflammation[3,4]. The mechanisms underlying epileptogenesis remain a significant area of inquiry, and the development of new effective treatments for epilepsy is imperative.

The highly selective α2 adrenoceptor agonist, dexmedetomidine (DEX), is commonly used for patient sedation in the intensive care unit. A growing body of research has demonstrated that DEX exhibits neuroprotective properties, including the reduction of inflammatory response and oxidative stress, inhibition of apoptosis, protection of the blood-brain barrier, maintenance of coagulation-anticoagulant system balance, and prevention of vasospasm[5,6]. Moreover, systemic administration of DEX can improve neurocognitive functions[7,8] and reduce neuroinflammation after various other conditions, including surgery[9,10]. However, the mechanism for this phenomenon has yet to be fully verified.

Methyl-CpG binding protein 2 (MeCP2) was initially identified as a conventional transcriptional repressor. However, current evidence suggests that MeCP2 plays a role in shaping the structural and functional characteristics of neurons during both neurodevelopment and adulthood[11]. Various neurodevelopmental disorders, such as Rett Syndrome (RTT; loss-of-function), autism spectrum disorder (ASD; reduced expression), fetal alcohol spectrum disorders (FASD; altered expression), MeCP2 duplication syndrome (MDS; gain-of-function), and severe neonatal encephalopathy, have been linked to MeCP2 mutations or altered expression[12,13].

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Furthermore, it has been reported that inhibition of MeCP2 expression eliminates abnormal EEG discharges in epileptic mice[14].

Mutations in the MeCP2 gene that are associated with disease have been found to impact the expression of a large number of genes[15]. One of these genes, brain-derived neurotrophic factor (BDNF), which is involved in regulating neuronal function, is crucial for neuronal survival, development, and adaptability[16]. Several research studies have established a connection between the malfunction of MeCP2 and impaired BDNF signaling[17,18]. The introduction of BDNF into the brains of mice with mutated MeCP2 has been shown to alleviate many of the physiological and behavioral deficits associated with Rett syndrome[17]. Furthermore, BDNF exerts its role in improving cognitive function mainly by activating intracellular signaling through specific binding to tyrosine receptor kinase B (TrkB), a highly expressed signaling receptor in the brain, which generates catalytic activity upon specific binding to BDNF. TrkB forms homodimers that trigger auto-phosphorylation of tyrosine residues in the cytoplasmic domain, which in turn activates downstream signaling pathways[19].

This research investigated the impact of DEX on hippocampal neuronal injury in neonatal rats and the regulation of MeCP2 and components of the BDNF/TrkB signaling pathway in a magnesium-free *in vitro* EP model. The aim was to offer comprehensive experimental support for the potential clinical use of DEX.

Materials and Methods

Experimental animals

Pregnant Sprague Dawley rats of Specific Pathogen-Free (SPF) grade were procured from Hunan SJA Laboratory Animal Co., LTD (Changsha, China). The rats, weighing 250±30 g, were housed in standard animal cages with three animals per cage and were provided with standard pellets as their diet. They were maintained in a pathogen-free facility under a 12-h light/dark cycle at a temperature of 22±1°C and a humidity of approximately 50%. The study protocol was approved by the Medical Ethics Committee of the Fourth Hospital of Changsha (CSSDSYY-YXLL-SC-2023-02-24).

Isolation and cultivation of hippocampal neurons in neonatal rats

The isolation of hippocampal neurons from neonatal rat brain tissues was conducted in accordance with the methodology outlined in a previous study[20,21]. Neonatal rats were euthanized within 24 h of birth through cervical dislocation following anesthesia with isoflurane. Subsequently, the neonatal rats were immersed in 75% ethanol for 2 min. The skin and skull of the rat's head were then removed, and the entire brain was extracted. The hippocampal tissue located beneath the cerebral cortex was carefully excised and transferred into pre-chilled sterile PBS. The hippocampal tissue was then dissected into small fragments using ophthalmic scissors. Following this, the hippocampal tissuewas added toa solution of 0.25% trypsin and 0.02% EDTA, and the tissue was subjected to enzymatic digestion at 37°C for 5 min to generate a single-cell suspension. After centrifugation to remove the supernatant, the cell pellet was resuspended in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS and 1% penicillin/streptomycin. The suspension was then cultured in a humidified incubator with 5% CO_2 at 37°C[22]. The hippocampal neurons were characterized through morphological examination and immunofluorescence (IF) analysis targeting neuron-specific enolase (NSE) and microtubule-associated protein 2 (MAP2)[20].

Cell experiment design

Following the removal of the maintenance culture medium, the neurons underwent three rinses with a magnesium-free external solution containing 2.5 mmol/L KCl, 145 mmol/L NaCl, 10 mmol/L glucose, 0.002 mmol/L glycine, 2 mmol/L calcium chloride, and 10 mmol/L HEPES at a pH of 7.4. Subsequently, the neurons were exposed to the magnesium-free external solution for a period of 3 h to initiate an *in vitro* EP model[23]. The targeting sequences of the small interfering-(si-) RNAs are shown in (Table 1).

Table 1 — The sequences related to this study

Item	Sequences (5′-3′)
si-NC Targeting sequence	TTAAGCCGGTTGCCCGGA
si-MeCP2 Targeting sequence	GCAGAGACATCAGAAGGGTCA
si-BDNF Targeting sequence	GAGCTGAGCGTGTGTGACAGTATTA
Forward primer of β-actin	ACATCCGTAAAGACCTCTATGCC
Reverse primer of β-actin	TACTCCTGCTTGCTGATCCAC
Forward primer of BDNF promoter	GGCTTCTGTGTGCGTGAATTTGC
Reverse primer of BDNF promoter	AAAGTGGGTGGGAGTCCACGAG

Experiment 1: Primary hippocampal neurons were randomly assigned to six groups: (1) Control group: Neurons were exposed to the standard culture medium for 3 h. (2) EP group: Neurons were exposed to the magnesium-free solution for 3 h. (3) EP+DEX1 group: Neurons were exposed to the magnesium-free solution for 3 h, followed by 1 μM DEX treatment for 2 h. (4) EP+DEX10 group: Neurons were exposed to magnesium-free solution for 3 h followed by 10 μM DEX treatment for 2 h. (5) EP+DEX100 group: Neurons were exposed to magnesium-free solution for 3 h followed by 100 μM DEX treatment for 2 h. (6) EP+DEX200 group: Neurons were exposed to magnesium-free solution for 3 h followed by 200 μM DEX treatment for 2 h. Following treatment with either the normal cell extract or magnesium-free extract, all groups were maintained in theculture medium for 24 h before subsequent experimentation[23].

Experiment 2: To assess the impact of MeCP2 on hippocampal neuronal damage induced by magnesium-free, neurons were divided into four groups: (1) EP+si-NC group: Neurons were exposed to magnesium-free solution for 3 h following transfection with si-NC. (2) EP+si-MeCP2 group: Neurons were exposed to the magnesium-free solution for 3 h following transfection with si-MeCP2. (3) EP+si-MeCP2+si-NC group: Neurons were exposed to the magnesium-free solution for 3 h following transfection with si-MeCP2 and si-NC. (4) EP+si-MeCP2+si-BNDF group: Neurons were exposed to the magnesium-free solution for 3 h following transfection with si-MeCP2 and si-BNDF.

Experiment 3: To evaluate the effect and underlying mechanism of DEX on hippocampal neuronal damage induced by magnesium-free, neurons were categorized into three groups: (1) EP+DEX100 group: Neurons were exposed to magnesium-free solution for 3 h followed by treatment with 100 μM DEX treatment for 2 h. (2) EP+DEX100+oe-NC group: Neurons were transfected with oe-NC and exposed to magnesium-free solution for 3 h, followed by treatment with 100 μM DEX treatment for 2 h. (3) EP+DEX100+oe-BNDF group: Neurons were transfected with oe-BNDF and exposed to magnesium-free solution for 3 h, followed by treatment with 100 μM DEX treatment for 2 h.

Cell transfection

To inhibit the expression of MeCP2 and BDNF, small interfering RNAs, rat si-MeCP2 (HG-RI051356, HonorGene, Changsha, China) and rat si-BDNF (HG-RI087634, HonorGene), were utilized. Hippocampal neurons were cultured in six-well plates and were then transfected with si-NC, si-MeCP2, and si-BDNF, respectively. To obtain the overexpression of MeCP2, oe-MeCP2 (pcDNA3.1-MeCP2-3xFlag, HG-RO051356, HonorGene) was used. The transfected cells were cultured in a medium containing puromycin to select stable cells. The oe-NC group was transfected with the empty vector. Subsequently, the neurons were used for the next experiments.

IF analysis

Hippocampal neurons were cultured in 24-well plates overnight. Subsequently, neurons were subjected to a 60-min blocking step at room temperature using a 5% BSA solution, after which they were incubated with anti-NSE antibody (66150-1-Ig, Proteintech, Rosemont, IL, USA) or anti-MAP2 antibody (ab11267, Abcam, Cambridge, MA, USA) at 4°C overnight. The neurons were then exposed to goat anti-mouse IgG (AWS0005c, Abiowell, Changsha, China) at 37°C for 1 h, and the nuclei were counterstained with DAPI (AWC0291a, Abiowell). Finally, neurons were visualized using a fluorescence microscope (BA210T, Motic, Xiamen, China).

Neuronal viability evaluation

Neuronal viability was assessed using cell counting kit-8 (CCK-8) assay (AWC0114a, Abiowell). 100 μL of suspended cells were seeded in a 96-well plate at a density of 5×10^3 cells/well, and each experimental group was subjected to the incubation mentioned above method. Following the experimental treatment, the culture medium was aspirated and replaced with 100 μL of fresh medium, and 10 μL of the CCK-8 reaction solution was added to each well. Subsequently, the absorbance at a wavelength of 450 nm was quantified using a microplate reader (MB-530, Heales, Shenzhen, China) after the culture plate had been incubated in a 5% CO_2 atmosphere at 37°C for 4 h.

Western blotting

The neuron samples were collected and subsequently lysed using RIPA lysate (AWB0136, Abiowell). Following centrifugation at a speed of 12000 rpm or 15 min, the liquid portion (supernatant) was carefully transferred into a 1.5 mL centrifuge tube. Proteins were separated using sodium dodecyl sulfate (SDS) gel electrophoresis and subsequently transferred onto nitrocellulose membranes. The membranes were enclosed within a blocking buffer solution comprising 5% skimmed milk. The membranes were subsequently incubated with primary antibodies overnight at a

temperature of 4°C. The primary antibodies utilized in this research were Bcl-2 (1: 1000, AWA43352, Abiowell), Bax (1: 1000, AWA47651, Abiowell), Cleaved-caspase3 (1: 1000, #9661, Cell Signaling Technology, Danvers, MA, USA), MeCP2 (1: 2000, AWA46149, Abiowell), BDNF (1: 1000, AWA44479, Abiowell), p-TrkB (1: 1000, AWA49009, Abiowell), and TrkB (1: 1000, AWA49013 Abiowell). Subsequently, the membranes underwent incubation with the secondary antibody HRP goat anti-rabbit IgG (1: 6000, SA00001-2, Proteintech) for 90 min at ambient temperature. Finally, the membranes underwent incubation with ECL chemiluminescence solution (AWB0005, Abiowell) for 1 min, followed by observation using the chemiluminescence imaging system (ChemiScope6100, CLiNX, Shanghai, China). The internal reference protein used in this study was β-actin (1: 5000, AWA80002, Abiowell). ImageJ software was employed to assess and compare the intensity of individual bands in western blotting experiments.

Enzyme-linked immunosorbent assay (ELISA) analysis

The levels of IL-6 and TNF-α in the culture supernatants were detected using the rat IL-6 ELISA kit (CSB-E04640r, CUSABIO, Wuhan, China) and rat TNF-α ELISA kit (CSB-E11987r, CUSABIO) following the manufacturer's instructions, respectively. The absorbance value at 450 nm was measured using a spectrophotometric microplate reader (MB-530, Heales).

Assessment of ROS and MDA levels and SOD activity

The generation of intracellular ROS was determined by ROS assay kit (S0033S, Beyotime, Nanjing, China)[24]. Hippocampal neurons and DCHF-DA reagent were co-incubated for 20 min in a cell culture incubator. The intracellular fluorescence intensity was assessed using a flow cytometer (A00-1-1102, Beckman Coulter, Miami, FL, USA). MDA and SOD assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to measure the level of MDA and the activity of SOD in the hippocampal neurons.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was conducted using a ChIP kit (ab500, Abcam). In brief, 1% formaldehyde was employed to induce crosslinking between the protein and DNA. Following the lysis of the neurons, chromatin was fragmented into small segments using micrococcal nuclease. Subsequently, 5 μg of antibody was utilized to immunoprecipitate the DNA-protein complex. This was followed by treatment with NaCl and proteinase K to reverse the DNA-protein crosslinking, and then DNA purification and recovery. The obtained DNA was subsequently subjected to qPCR for further analysis. The primers related to this work are listed in (Table 1). The fold enrichment was calculated after normalization to the 1% input.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

The TUNEL assay kit (40306ES50, Yeasen, Shanghai, China) was utilized to detect the apoptosis of hippocampal neurons[25]. Neurons were initially treated with 4% paraformaldehyde for 30 minutes to fix them and then permeabilized with 0.2% Triton X-100 for another 30 min. Following this, the neurons were exposed to TUNEL reaction solution and incubated for 1 h at 37°C. DAPI solution was then added, and the reaction was carried out for 10 min at room temperature. Afterward, the neurons were rinsed with PBS three times for 5 min each and mounted with neutral resin. The apoptotic neurons were visualized using a fluorescence microscope (BA410T, Motic), and the percentage of TUNEL-positive neurons was determined.

Statistical analysis

All data were presented as the mean ± SD. Statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The *t*-test was employed to compare two groups, whereas one-way analysis of variance (ANOVA) was utilized to compare multiple groups. $P< 0.05$ was considered statistically significant.

Results

Culture and identification of hippocampal neurons in vitro

Initially, the structural characteristics of hippocampal neurons obtained from the brain tissues of rats born within 24 h were observed under a microscope. As shown in Figure 1A, neurons exhibited slenderness. This morphology is consistent with the characteristics of hippocampal neurons[26]. Moreover, the IF staining results demonstrated that the expression of NSE and MAP2 was notably induced (Fig. 1B). Given that NSE and MAP2 serve as pivotal biomarkers in hippocampal neurons[27], these findings indicated that the isolation of hippocampal neurons from rat brain tissues was successful.

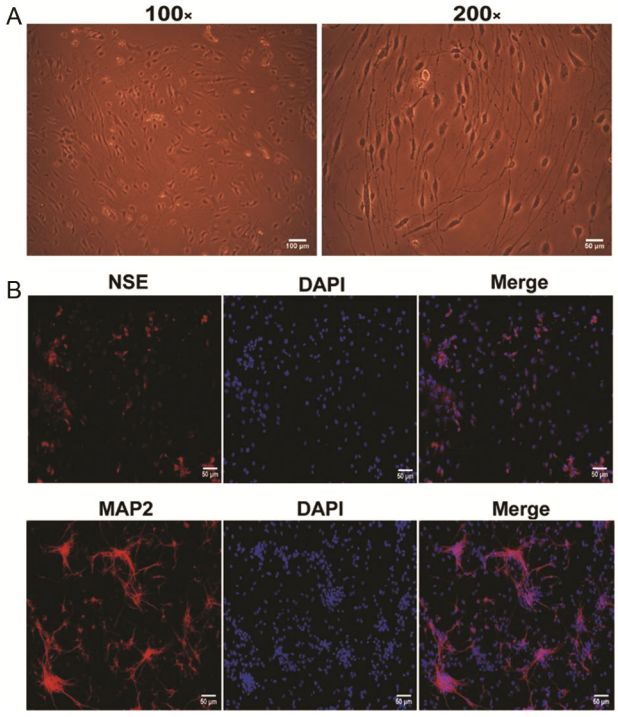

Fig. 1 — Culture and identification of hippocampal neurons *in vitro*. (A) The morphology of hippocampal neurons was observed using a light microscope at 100× and 200× magnifications; and (B) The IF staining was employed to detect the expression of NSE and MAP2, with red fluorescence indicating the presence of NSE or MAP2, and blue fluorescence indicating nuclear staining using DAPI

DEX alleviated hippocampal neuronal damage

Next, we evaluated the impact of DEX on the viability of hippocampal neurons by CCK-8 assay. The results indicated no significant difference in the EP+DEX1 and EP+DEX10 groups when compared to the EP group. However, a marked enhancement in hippocampal neuronal activity was evident following treatment with elevated concentrations of DEX (≥100 μM). Notably, there was no significant distinction between the EP+DEX100 group and the EP+DEX200 group (Fig. 2A). Moreover, western blotting results of Bcl-2, Bax, and Cleaved caspase-3 revealed that treatment with 100 μM and 200 μM DEX markedly inhibited neuronal apoptosis induced by magnesium-free (Fig. 2B). We also found that the levels of pro-inflammation cytokines, IL-6 and TNF-α, were significantly decreased in EP+DEX100 and EP+DEX200 groups (Fig. 2C & D). Additionally, the levels of ROS and MDA, and the activity of SOD, indicators of oxidative stress[28,29], were evaluated in order to investigate the effect of DEX on oxidative stress of hippocampal neurons. The data revealed that EP significantly elevated the contents of ROS and MDA and reduced the activity of SOD, while 100 μM and 200 μM DEX restored these changes (Fig. 2E & F). Furthermore, previous studies have reported that MeCP2, a key neurodevelopmental gene, played a crucial role in EP[30,31]. Therefore, we then evaluated the expression of MeCP2 in hippocampal neurons. As expected, EP notably induced the expression of MeCP2. However, DEX significantly inhibited it (Fig. 2G). These findings indicated that DEX of 100 μM and 200 μM alleviated hippocampal neuronal damage induced by magnesium-free, and this effect might be through MeCP2. Additionally, there was no significant difference between the treatment of 100 μM DEX and 200 μM DEX. Based on the above data, 100 μM of DEX was selected for further investigation.

MeCP2 inhibited the BDNF/TrkB signaling in the EP model in vitro

Targeting BDNF/TrkB pathway was widely recognized to prevent or suppress EP[32]. Therefore, we subsequently explored whether MeCP2 exerts effects on EP through the BDNF/TrkB pathway. As displayed in Figure 3A, after MeCP2 silencing, the expression of MeCP2 was reduced, whereas the expression of BDNF and p-TrkB was remarkably induced. However, the expression of BDNF and p-TrkB were restored when BDNF was silenced. Next, ChIP results showed enrichment of MeCP2 in the promoter region of the BDNF gene (Fig. 3B), which indicated that MeCP2 might inhibit BDNF/TrkB expression through binding to the BDNF promoter.

MeCP2 silencing alleviated hippocampal neuronal damage through upregulating BDNF

To further explore the molecular mechanism of MeCP2 on hippocampal neuronal damage in EP, we next transfected si-MeCP2 and si-BDNF to hippocampal neurons, respectively. As shown in Figure 4A, MeCP2 silencing significantly increased neuronal viability, while it was reduced after BDNF was silenced. Moreover, in the EP+si-MeCP2 group, the upregulation of Bcl-2 expression was found to be statistically significant. In contrast, the expression levels of Bax and Cleaved-caspase3 were observed to be significantly reduced, which suggested that MeCP2 silencing significantly inhibited neuronal apoptosis. However, BDNF silencing restored these changes (Fig. 4B). Additionally, TUNEL staining data displayed consistent results (Fig. 4C). The levels of IL-6 and TNF-α were also remarkably down-regulated after MeCP2 was silenced. However, they were significantly

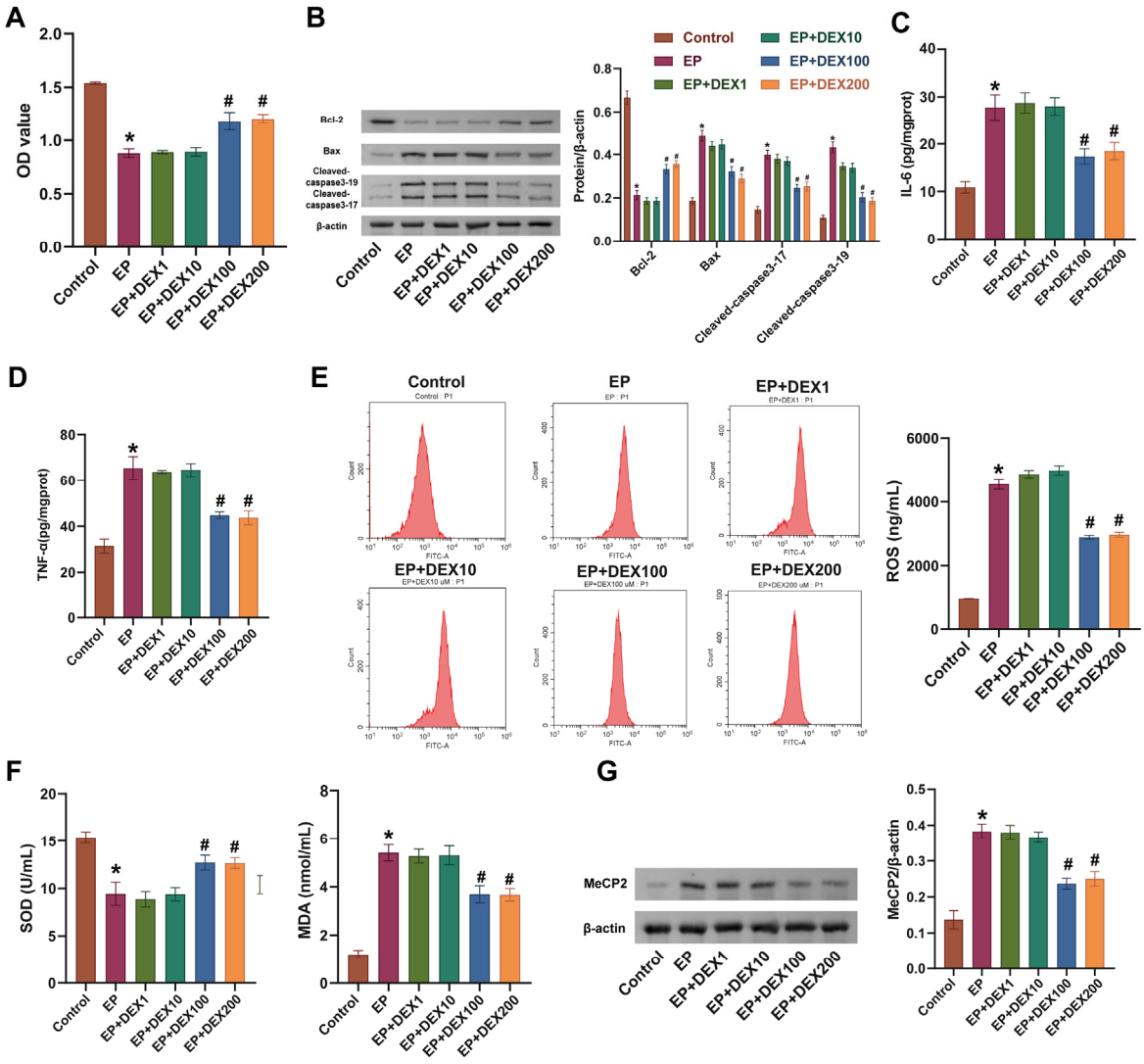

Fig. 2 — High concentrations of DEX elevated neuronal viability as well asreduced apoptosis, inflammation, oxidative stress, and MeCP2 expression. (A) The neuronal viability was evaluated by CCK-8; (B) The expression of Bcl-2, Bax, and Cleaved-caspase3 were assessed; (C-D) The levels of IL-6 and TNF-α in the culture supernatants were evaluated. (E) The levels of ROS were evaluated using flow cytometry; (F) The MDA content and SOD activity in the hippocampal neurons were assessed; and (G) The expression of MeCP2 was assessed. n = 3 per group. $^{*}P < 0.05$ *vs.* Control, $^{\#}P < 0.05$ *vs.* EP

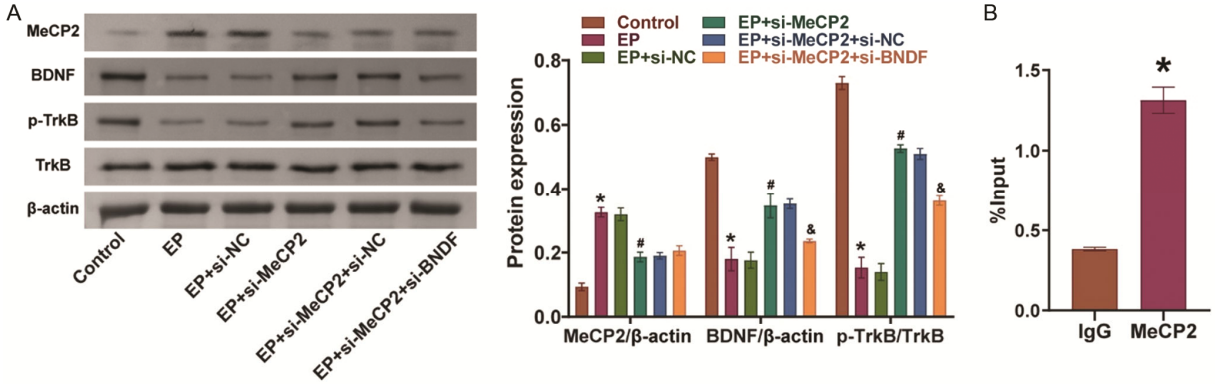

Fig. 3 — MeCP2 inhibited the BDNF/TrkB signaling in the EP model *in vitro*. (A) The expression of MeCP2, BDNF, p-TrkB, and TrkB was analyzed. $^{*}P < 0.05$ *vs.* Control, $^{\#}P < 0.05$ *vs.* EP+si-NC, $^{\&}P < 0.05$ *vs.* EP+si-MeCP2+si-NC; and (B) ChIP assay was utilized to assess the enrichment of MeCP2 at the promoter of BDNF. n = 3 per group. $^{*}P < 0.05$ *vs.* IgG

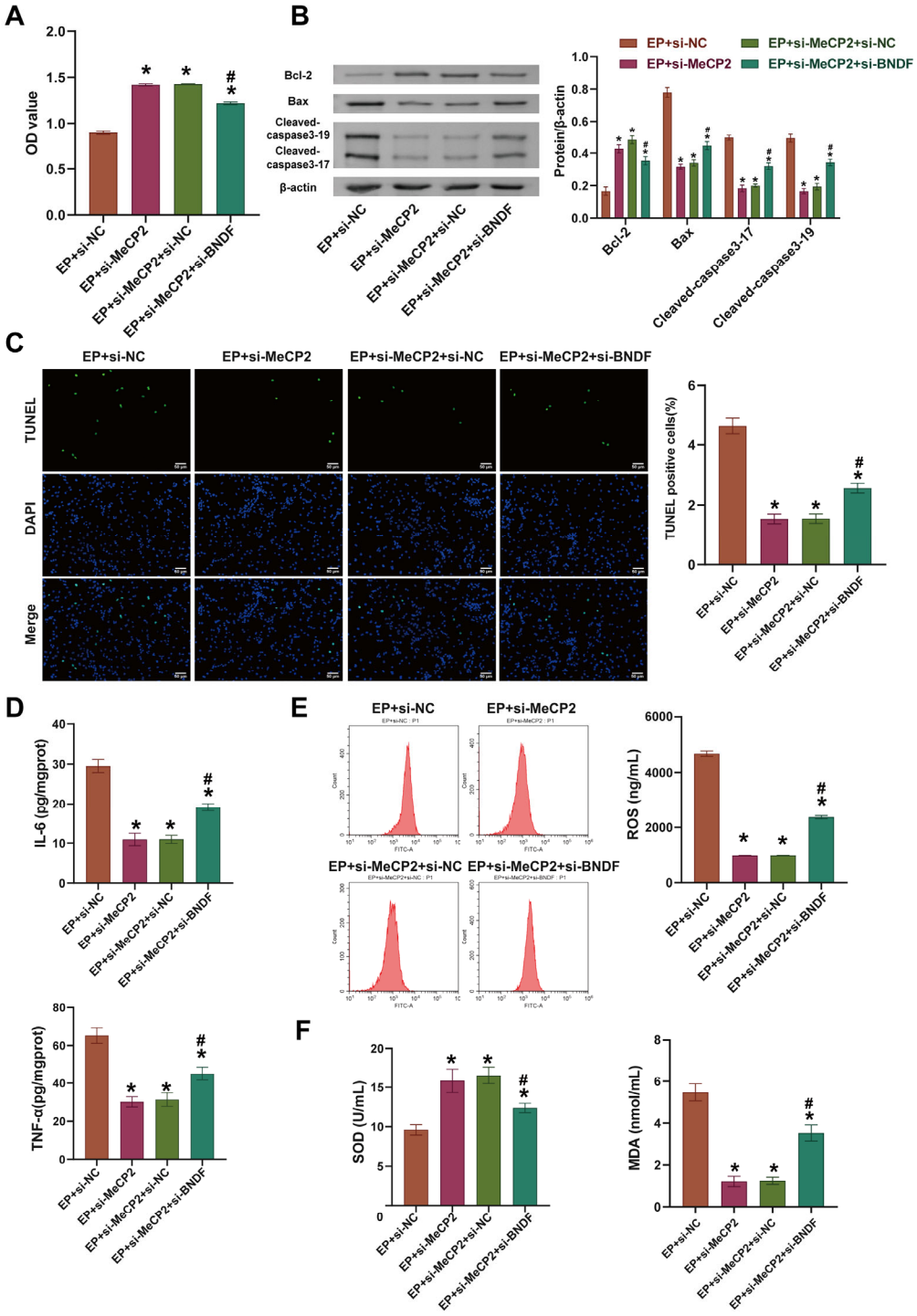

Fig. 4 — MeCP2 silencing alleviated hippocampal neuronal damage through upregulating BDNF. (A) The neuronal viability was evaluated by CCK-8; (B) The expression levels of Bcl-2, Bax, and Cleaved caspase3 were evaluated; (C) Represented images of TUNEL staining in hippocampal neurons; (D) The levels of IL-6 and TNF-α in the culture supernatants were evaluated; (E) The levels of ROS were evaluated using flow cytometry; and (F) The MDA content and SOD activity in the hippocampal neuron were assessed. n = 3 per group.$^{*}P< 0.05$ *vs.* EP+si-NC, $^{\#}P< 0.05$ *vs.* EP+si-MeCP2+si-NC

increased in the EP+si-MeCP2+si-BDNF group (Fig. 4D). Furthermore, MeCP2 silencing also notably inhibited oxidative stress of hippocampal neurons. Nevertheless, compared to the EP+si-MeCP2+si-NC group, BDNF silencing significantly promoted oxidative stress (Fig. 4E & F). The above data indicated that MeCP2 silencing alleviated magnesium-free-induced hippocampal neuronal damage by upregulating BDNF.

DEX alleviated hippocampal neuronal damage through MeCP2/BDNF/TrkB signaling pathway

Based on the above results, we found that DEX inhibited hippocampal neuronal damage and MeCP2 expression. In addition, MeCP2 silencing alleviated hippocampal neuronal damage through the BDNF/TrkB pathway. Therefore, we hypothesized that DEX may exert therapeutic effects on hippocampal neurons through the MeCP2/BDNF/TrkB signaling pathway. MeCP2 was then overexpressed in the DEX-treated EP model. As expected, the expression of MeCP2 was increased, while the expression of BDNF and p-TrkB/TrkB was decreased after MeCP2 was overexpressed (Fig. 5A). Moreover, compared to the EP+DEX100+oe-NC group, MeCP2 overexpression significantly reduced neuronal viability in EP (Fig. 5B). Additionally, based on western blotting results of apoptosis-related proteins and TUNEL staining results, MeCP2 overexpression remarkably promoted hippocampal neuronal apoptosis (Fig. 5C & D, and Suppl. Fig. S1). Compared with the EP+DEX100+oe-NC group, the levels of IL-6 and TNF-α were significantly upregulated after MeCP2 was overexpressed (Fig. 5E), indicating that MeCP2 promoted neuroinflammation in EP. Furthermore, the contents of ROS and MDA were significantly increased, and the activity of SOD was significantly decreased in the EP+DEX100+oe-MeCP2 group (Fig. 5F & G). Taken together, DEX alleviated hippocampal neuronal damage by down-regulating MeCP2 expression through BDNF/TrkB signaling pathway.

Discussion

DEX has been demonstrated to have a significant impact on the pathogenesis of various diseases, including EP[33-35]. In this investigation, we utilized hippocampal neurons subjected to magnesium deprivation as an *in vitro* model of EP, consistent with previous literature[36,23]. Our findings indicated that DEX notably facilitated the growth of hippocampal neurons and suppressed apoptosis in the absence of magnesium. However, prior research has indicated that DEX impeded the proliferation, invasion, and migration of diverse malignant tumors[37]. This inconsistency may be attributed to variations in the underlying disease pathologies. Furthermore, a randomized controlled trial conducted by Ashish *et al.* revealed that lower perioperative values of S100b were observed in patients with temporal lobe EP surgery after receiving intraoperative DEX, suggesting that DEX may play a role in cerebroprotection during EP surgery[38]. It has been reported that DEX exerted its anti-seizure effects *via* α2-adrenergic receptors[39]. Similarly, our results validated the biological role and molecular mechanism of DEX, suggesting that DEX might be a novel and effective anti-seizure medication in EP.

MeCP2 is a key regulator in EP progression, which regulates neurodevelopmental and cognitive functions[40,31]. Moreover, it has been acknowledged as having a crucial function in the regulation of activity-dependent gene transcription, which is significant for neural function, spine density maturation, neuronal connectivity, dendritic arborization, behavior, and various types of synaptic plasticity[41,42]. In our study, there was a remarkable increase in MeCP2 expression after magnesium-free treatment, while DEX could restore it. Therefore, we hypothesized that DEX may exert a therapeutic effect on hippocampal neuron damage through MeCP2 and then centered on investigating the involvement of MeCP2 in the progression of EP. In our investigation, inhibition of MeCP2 promoted neuronal viability and reduced apoptosis, inflammation, and oxidative stress, which indicates that MeCP2 silencing significantly alleviated hippocampal neuronal damage induced by magnesium-free.

The BDNF/TrkB pathway has been observed to undergo rapid activation in the hippocampus following an episode of status epilepticus[32]. Meanwhile, MeCP2-mechanism in the regulation of neurotrophic factor expression has been reported in various cellular contexts. Huang *et al.* have reported that the excessive expression of MeCP2 suppressed the transcription of BDNF and the activation of the TrkB signaling in neuro-2a cells[43]. Moreover, MeCP2 knockout mice have down-regulated expression of BDNF in the hippocampus[44,45]. Additionally, we found a binding of MeCP2 and the BDNF promoter utilizing ChIP in our study. Therefore, we inferred that MeCP2 silencing exerts therapeutic effects through the BDNF/TrkB pathway. As expected, our findings revealed that

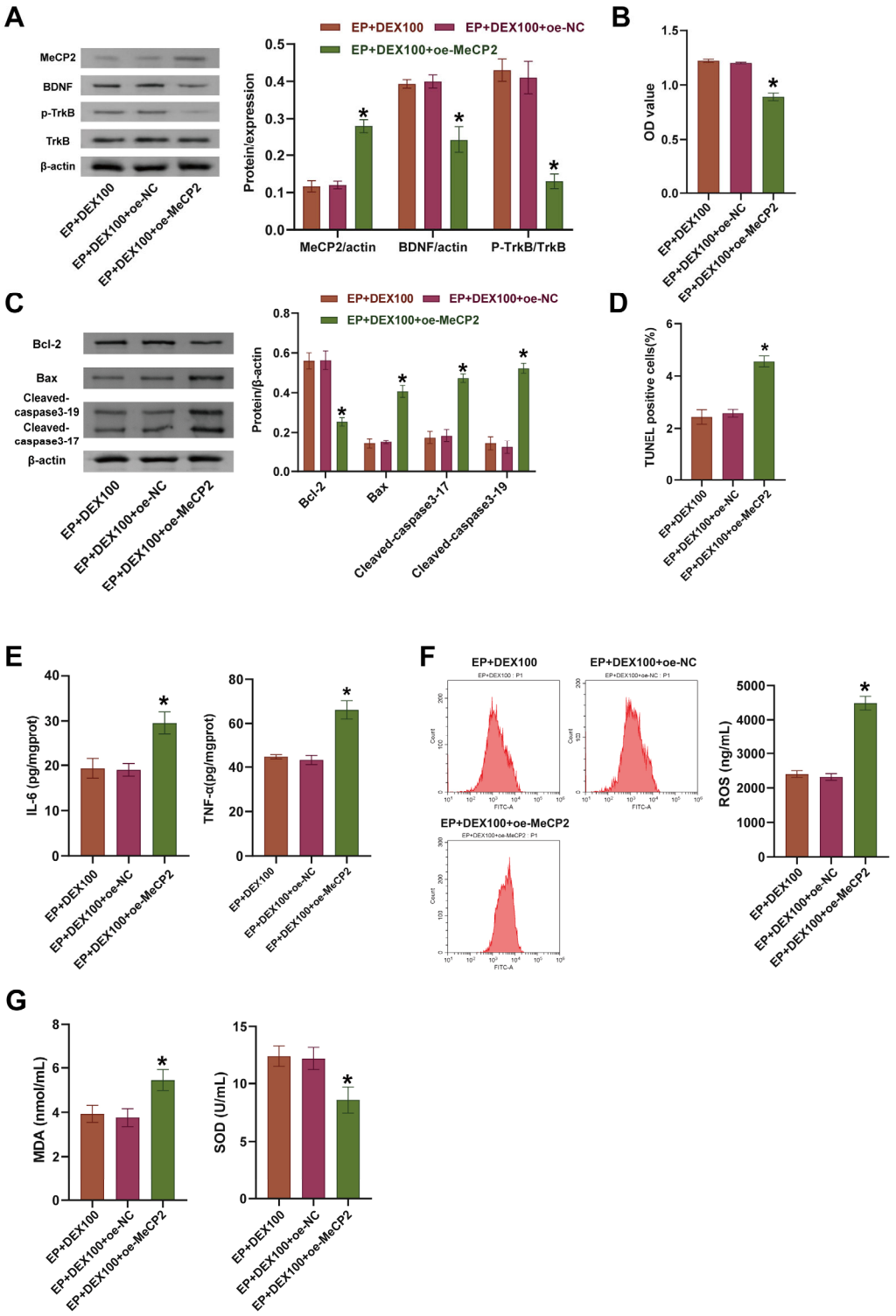

Fig. 5— DEX alleviated hippocampal neuronal damage through the MeCP2/BDNF/TrkB signaling. (A) The levels of MeCP2, BDNF, and p-TrkB/TrkB were evaluated in hippocampal neurons; (B) The neuronal viability was evaluated; (C) The expression levels of Bcl-2, Bax, and Cleaved-caspase3 were assessed; (D) The percentage of TUNEL-positive cells in hippocampal neurons; (E) The levels of IL-6 and TNF-α in the culture supernatants were evaluated; (F) The levels of ROS were evaluated using flow cytometry; and (G) The MDA content and SOD activity in the hippocampal neuron were assessed. n = 3 per group. $^{*}P< 0.05$ *vs.* EP+DEX100+oe-NC

BDNF silencing restored the increased neuronal viability and reduced apoptosis, inflammation, and oxidative stress, which were induced by MeCP2 silencing. Nevertheless, MeCP2 could also be functioning through other parallel pathways. For example, Zhou *et al.* found that a complex involving the transcription factor 20 (TCF20) was found to interact with MeCP2 at the chromatin level. Partial restoration of TCF20 levels was observed to alleviate the behavioral impairments resulting from MeCP2 overexpression, indicating a functional association between MeCP2 and TCF20 in the pathogenesis of MeCP2 duplication syndrome[46].

In the current investigation, cultured hippocampal neurons were subjected to a magnesium-depleted medium to simulate seizure-like activity *in vitro*, with the aim of elucidating the underlying mechanism of DEX in epileptogenesis. While this *in vitro* model has been commonly utilized to probe the fundamental molecular pathways of epileptogenesis, it is important to note that the cultured hippocampal neurons *in vitro* did not establish structural connections or manifest cerebral functions, nor did they exhibit the clinical seizures characteristic of individuals with epileptic conditions[47]. Consequently, our findings pertaining to the role and molecular mechanism of DEX in modulating seizure activity may not entirely mirror the molecular mechanism of DEX underlying the clinical epileptic state. Further animal and clinical research is imperative in order to acquire a more profound comprehension of the fundamental mechanisms.

Conclusion

DEX had significant neuroprotective effects in magnesium-free-treated hippocampal neurons. The observed effects may stem from a decrease in MeCP2 expression, leading to the activation of the BDNF/TrkB signaling pathway. Our study contributes to a deeper comprehension of the neurological impact of DEX and highlights its potential as a therapeutic agent for addressing EP and related neurological conditions.

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Conflict of interest

All authors declare no conflict of interest.

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